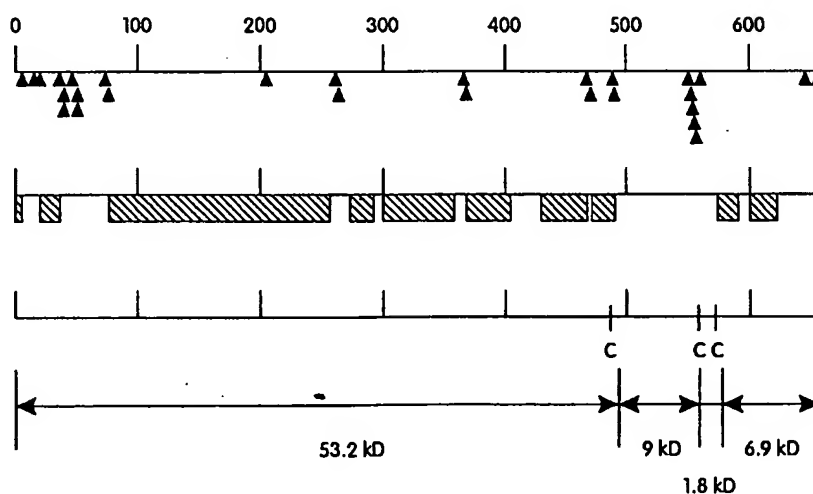


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(54) Title: NOVEL MALIGNANT CELL TYPE MARKERS OF THE INTERIOR NUCLEAR MATRIX**(57) Abstract**

Disclosed are genetic sequences and their encoded amino acid sequences for two interior nuclear matrix proteins useful as markers of malignant cell types. Primary and secondary structure analysis of the proteins is presented as well as means for their recombinant production, and compositions and methods for the use of these markers in clinical assays and cancer therapies.

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Novel Malignant Cell Type Markers
of the Interior Nuclear Matrix

Background of the Invention

All eucaryotic cells, both plant and animal, have a nucleus surrounded by the cell cytoplasm. The nucleus contains the cellular DNA complexed with protein and termed chromatin. The chromatin, with its associated
5 proteins, constitutes the major portion of the nuclear mass and is organized by the internal protein skeleton of the nucleus, referred to here as the nuclear matrix (NM). The nuclear matrix also is defined as the nuclear structure that remains following removal of the
10 chromatin by digestion with DNase I and extraction with high salt. This skeletal nuclear structure further is characterized by the "interior nuclear matrix" (INM) and the bounding nuclear pore-lamina complex.

15 Diverse studies have implicated the NM in a wide variety of nuclear functions fundamental to the control of gene expression (For a general review see, for example, Fey et al. (1991) Crit. Rev. Euk. Gene Express 1:127-143). In particular, as described in
20 U.S. Pat. Nos. 4,882,268 and 4,885,236, it is now known that certain nuclear matrix proteins, specifically interior nuclear matrix proteins, are useful as marker proteins for identifying cell types. For example, the presence and abundance of particular INM proteins have
25 been shown to be characteristic of specific cell types and can be used to identify the tissue of origin of a cell or cell fragment present in a sample. One particularly important application of this discovery is the use of marker INM proteins in evaluating metastatic

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tissue. It is also known that the expression of certain INM proteins is altered in malignant or otherwise dysfunctional cells. The altered expression pattern of these proteins in malignant and/or
5 dysfunctioning cells also makes the proteins and nucleic acids encoding the proteins useful as marker proteins, alone or in combination, for diagnostic purposes and for evaluating tissue viability. US Pat. Nos. 4,882,268 and 4,885,236, issued 11/21/89 and
10 12/5/89, respectively, to Penman and Fey, disclose a method for selectively extracting insoluble INM proteins and their associated nucleic acids from cells or cellular debris and distinguishing the expression pattern of these proteins in a particular cell type by
15 displaying the proteins on a two-dimensional electrophoresis gel. In addition, it recently has been discovered that INM proteins or protein fragments also may be released in soluble form from dying cells. (US Application Serial No. 785,804, filed October 31,
20 1991.)

To date, molecular characterization of the specific proteins of the NM, particularly the INM, remain poorly defined due to the low abundance of these proteins in
25 the cell and their generally insoluble character. The ability to isolate and characterize specific nuclear matrix proteins and the genetic sequences encoding them at the molecular level is anticipated to enhance the use of these proteins and their nucleic acids as marker
30 molecules, and to enhance elucidation of the biological role of these proteins in vivo.

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It is an object of this invention to provide genetic sequences encoding INM proteins useful as markers of malignant cell types. Another object is to provide enhanced means for identifying these proteins
5 and their nucleic acids, including RNA transcripts, in samples. Yet another object of this invention is to provide compositions for use in diagnostic and other tissue evaluative procedures. Still another object is to provide genetic and amino acid sequences useful as
10 target molecules in a cancer therapy. These and other objects and features of the invention will be apparent from the description, figures and claims which follow.

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Summary of the Invention

Molecular characterization data, including DNA sequence data, for two INM proteins now have been
5 derived from an expression library, using monoclonal antibodies for these proteins. The proteins, designated herein as MT1 and MT2, are present at elevated levels in malignant tissue and extracellular fluids. Accordingly, the proteins and the genetic
10 sequences encoding them are thought to be useful as marker molecules for identifying tissue tumorigenesis in cell or body fluid samples.

Full or partial clones of the genes encoding these
15 proteins now have been isolated, and the DNA sequence, reading frames and encoded amino acid sequences of these DNAs determined. The DNA sequence for MT2 corresponds to the sequence disclosed by Yang, et al. (1992) J. Cell Biol. 116:1303-1317, and Compton et al.
20 (1992) J. Cell Biol. 116:1395-1408, referred to therein as NuMA. The nucleic acid (and the encoded amino acid sequence) described herein for MT1 has not been described previously and also constitutes a novel sequence sharing little sequence homology with those
25 sequences known in the art. In addition, MT1 has been subcloned into an expression vector, and the protein expressed as a cleavable fusion protein in E. coli. Both the MT1 and MT2 (NuMA) proteins are distributed throughout the nucleus (with the exception of the
30 nucleolus) in non-mitotic cells, and localize to the spindle during mitosis, as determined immunofluorescence.

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The genetic sequences described herein provide a family of proteins for each of the proteins MT1 and MT2, including allelic and species variants of MT1 and MT2. The family of proteins include these proteins
5 produced by expression in a host cell from recombinant DNA, the DNA itself, and the host cells harboring and capable of expressing these nucleic acids. The recombinantly produced proteins may be isolated using standard methodologies such as affinity chromatography
10 to yield substantially pure proteins. As used herein, "substantially pure" is understood to mean substantially free of undesired, contaminating proteinaceous material.

15 The family of proteins defined by MT1 includes proteins encoded by the nucleic acid sequence of Seq. ID No. 1, including analogs thereof. As used herein, "analog" is understood to include allelic and species variants, and other naturally-occurring and engineered
20 mutants. These variants include both biologically active and inactive forms of the protein. Particularly envisioned are DNAs having a different preferred codon usage, those having "silent mutations" of the DNA of Seq. ID No.1, wherein the changes in the genetic
25 sequence do not affect the encoded amino acid sequence, and DNAs encoding "conservative" amino acid changes, as defined by Dayoff et al., Atlas of Protein Sequence and Structure; vol. 5, Supp. 3, pp 345-362 (M.O. Dayoff, ed., Nat'l Biomed. Research Foundation, Washington,
30 D.C. 1979.)

Accordingly, the nucleic acids encoding the protein family of MT1 may be defined as those sequences which hybridize to the DNA sequence of Seq. ID No.1 under
35 stringent hybridization conditions. As used herein,

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stringent hybridization conditions are as defined in
Molecular Cloning: A Laboratory Manual, Maniatis,
et al. eds., Cold Spring Harbor Press, 1985, e.g.:
hybridization in 50% formamide, 5x Denhardt's Solution,
5 5 x SSPE, 0.1% SDS and 100 µg/ml denatured salmon
sperm, and washing in 2 x SSC, 0.1% SDS, at 37°C, and
1 x SSC, 0.1% SDS at 68°C.

The family of proteins defined by MT2 includes
10 proteins encoded by the nucleic acid sequence of Seq.
ID No. 3, including analogs thereof, including allelic
and species variants, and other naturally-occurring and
engineered mutants. These variants include both
biologically active and inactive forms of the protein.
15 Particularly envisioned are DNAs having silent
mutations, other preferred codon usages, and DNAs
encoding conservative amino acid changes. The nucleic
acids encoding the protein family of MT2 of this
invention may be defined as those sequences which
20 hybridize with the DNA sequence of Seq. ID No. 3 under
stringent hybridization conditions.

In another aspect, the invention provides nucleic
acid fragments ("oligonucleotides" or "oligomers")
25 which hybridize to genetic sequences encoding MT1, but
which do not necessarily encode functional proteins
themselves. The oligonucleotides include probes for
isolating genetic sequences encoding members of the MT1
family of proteins from a cDNA or genomic DNA library,
30 and/or for identifying genetic sequences naturally
associated with the MT1 protein coding sequence e.g.,
sequences lying upstream or downstream from the coding
sequences. For example, where the nucleic acid
fragment is to be used as a probe to identify other
35 members of the MT1 family, the nucleic acid fragment

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may be a degenerate sequence as described in Molecular Cloning: A Laboratory Manual, Maniatis, et al. eds., Cold Spring Harbor Press, 1985, designed using the sequence of Seq. ID No.1 as a template. Accordingly,
5 the oligonucleotide or nucleic acid fragment may comprise part or all of the DNA sequence of Seq. ID No. 1, or may be a biosynthetic sequence based on the DNA sequence of Seq. ID No. 1. The oligonucleotide preferably is suitably labelled using conventional
10 labelling techniques.

The oligonucleotides also include sequences which hybridize with the mRNA transcript encoding the MT1 protein. These complementary sequences are referred to
15 in the art and herein as antisense sequences. Antisense sequences may comprise part or all of the sequence of Seq. ID No. 1, or they may be biosynthetic sequences designed using the sequence of Seq. ID No. 1 as a template.

20

In still another aspect, the invention provides oligonucleotides which hybridize to the genetic sequences encoding members of the MT2 protein family. The fragments include antisense sequences and sequences
25 useful as probes for identifying members of the MT2 family and/or for identifying associated noncoding sequences. The hybridizing nucleic acids may comprise part or all of the sequence of Seq. ID No. 3 or may be biosynthetic sequences designed using the DNA sequence
30 of Seq. ID No. 3 as a template, preferably suitably labelled using conventional techniques.

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The genetic sequences identified herein encode proteins identified as marker proteins indicative of a malignancy or other cellular dysfunction in a tissue. Thus, in another aspect, the invention provides

5 compositions for obtaining antibodies useful for detecting cancer marker proteins in a sample using the proteins described herein in combination with a suitable adjuvant. In another aspect, the invention provides genetic templates for designing sequences

10 which hybridize specifically with the mRNA transcripts encoding these proteins. In still another aspect, the invention provides isolated DNA sequences for use in expressing proteins and protein fragments for the design of binding proteins, including antibodies, which

15 interact specifically with an epitope on MT1 or MT2. The invention also provides methods for evaluating the status of a tissue using the genetic sequences described herein, and the marker proteins encoded by them. Finally, the invention provides methods for

20 treating a malignancy in an individual using these marker proteins, or the genetic sequences encoding them, as target molecules to inhibit or disable the cell's ability to undergo cell division.

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Brief Descriptions of the Drawings

Fig. 1A-1D is a schematic representation of the
5 amino acid sequence of MT1 of Seq. ID No.1, showing:

- Fig. 1A: the location of the proline residues;
Fig. 1B: the areas defined as α -helices within the
sequence;
10 Fig. 1C: the location of the cysteine residues;
and
Fig. 1D: the sites of cleavage by NTCB;

Fig. 2A-2B is a schematic representation of the
15 amino acid sequence of MT2 of Seq. ID No.3, showing:

- Fig. 2A: the location of proline residues; and
Fig. 2B: the areas defined as α -helices within the
sequence;

20

Fig. 3: lists the levels of body fluid-soluble
MT2 and MT2-associated protein quantitated in various
normal and malignant tissue sample supernatants; and

- 25 Fig. 4: lists the levels of body fluid-soluble
MT2 and MT2-associated protein quantitated in sera
isolated from cancer patients and normal blood donors.

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Detailed Description

In an attempt to characterize INM proteins useful as malignant cell markers in biological assays, the genetic sequences encoding two INM proteins, herein referred to as MT1 and MT2, now have been identified and characterized. DNA sequences encoding these proteins now have been cloned by probing expression libraries using monoclonal antibodies raised against the isolated INM proteins MT1 and MT2. The proteins were isolated from malignant cells essentially following the method of Penman and Fey, described in U.S. Pat. Nos. 4,882,268 and 4,885,236, the disclosures of which are herein incorporated by reference. The cloned DNAs, then were sequenced and their reading frames identified and analyzed. The genetic sequence encoding MT2 also has been disclosed by others (Yang, et al. (1992) J. Cell Biol. 116:1303-1317 and Compton et al. (1992) J. Cell. Biol. 116:1395-1408), and is referred to by them as "NuMA". Comparison of MT1 and MT2 (NuMA) with other sequences in the art indicate that the sequences encoding these proteins constitute sequences sharing little homology with previously described sequences.

25

MT1 also has been expressed as a cleavable fusion protein in E. coli and compared with the protein isolated from mammalian cells. Anti-MT1 antibodies raised against the natural-sourced MT1 protein also crossreact with the recombinantly produced protein.

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Both the natural-sourced and recombinantly produced proteins have the same apparent molecular weight when analyzed by SDS-PAGE (90kD), equivalent pI values (5.4), and both proteins show the same cleavage pattern when cleaved with 2-nitro-3-thiocyanobenzoic acid (NTCB, see infra.)

Immunolocalization data on MT1 indicates that MT1 protein is distributed within the INM in non-mitotic cells as discrete punctate foci, nonuniformly distributed throughout the nucleoplasm of the INM. Specifically, the foci are present in the interchromatinic regions of the nucleus and are distributed in a stable association that remains after chromatin extraction, as is anticipated for an interior nuclear matrix protein. In addition, MT1 foci are excluded from the nucleolus and the nuclear lamina. Moreover, during mitosis, the distribution of MT1 changes and MT1 becomes aligned in a stellate or star-shaped pattern at the spindle of the dividing cell. The protein does not co-localize with the chromosomes, suggesting that MT1 may play a structural role during mitosis. The immunolocalization data is consistent with the MT1 amino acid sequence analysis data which fails to find structural homology with any known DNA binding motifs, such as the "leucine zipper."

While the MT2 (NuMA) protein has not yet been recombinantly expressed, the predicted molecular weight of 238 kDa for this protein, calculated from the predicted amino acid sequence (see Seq. ID No. 3), agrees with that of the natural-sourced material.

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Immunolocalization studies on MT2 (NuMA) indicate that it also forms punctate foci located throughout the nucleoplasm of the non-mitotic cell, and also is excluded from the nucleolus. During mitosis the protein appears to migrate to the spindle poles of the dividing cell. The primary sequence appears to suggest a coiled-coil motif for the folded protein (Compton, et al. (1992) J. Cell Biol. 116:1395-1408; Yang, et al. (1992) J. Cell Biol. 116:1303-1317.)

10

I. How to Use

The nucleic acids disclosed herein encode proteins originally identified as marker proteins useful for identifying cell malignancies or other cell abnormalities. Specifically, significantly elevated levels of these proteins are detected in malignant cells and in extracellular fluids, e.g., sera, of cancer patients. (See PCT publication WO93/09437 and infra.) For example, the presence and/or abundance of these proteins or their transcripts in a sample containing cells or cell nuclear debris may be used to determine whether a given tissue comprises malignant cells or cells having other abnormalities, such as chromosomal abnormalities. The sample may be an exfoliated cell sample or a body fluid sample, e.g., a sample comprising blood, serum, plasma, urine, semen, vaginal secretions, spinal fluid, saliva, ascitic fluid, peritoneal fluid, sputum, tissue swabs, and body exudates such as breast exudate.

In addition, because INM proteins are released in soluble form from dying cells, the marker molecules may be used to evaluate the viability of a given tissue. For example, the marker proteins may be used to

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evaluate the status of a disease or the efficacy of a therapeutic treatment or procedure, by monitoring the release of these marker molecules into a body fluid over a period of time. Particularly useful body fluids

5 include blood, serum, plasma, urine, semen, vaginal secretions, spinal fluid, saliva, ascitic fluid, peritoneal fluid, sputum, tissue swabs, and body exudates such as breast exudate. Methods for performing these assays are disclosed in U.S. Pat.

10 Nos. 4,882,268 and 4,885,236 and in co-pending U.S. application Serial Nos. 214,022, filed June 30, 1988 and U.S. application Serial No. 785,804, filed October 31, 1991, the disclosures of which all are herein incorporated by reference.

15

All of these assays are characterized by the following general procedural steps:

1) detecting the presence and/or abundance of

20 the marker protein or its transcript in "authentic" or reference samples;

2) detecting the presence and/or abundance of the marker protein or its transcript in the sample of

25 interest; and

3) comparing the quantity of marker protein or its transcript in the sample of interest with the quantity present in the reference sample.

30

Where the assay is used to monitor tissue viability, the step of detecting the presence and abundance of the marker protein or its transcript in samples of interest is repeated at intervals and these

35 values then are compared, the changes in the detected

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concentrations reflecting changes in the status of the tissue. Where the assay is used to evaluate the efficacy of a therapy, the monitoring steps occur following administration of the therapeutic agent or
5 procedure (e.g., following administration of a chemotherapeutic agent or following radiation treatment.)

It is not required that the selected marker protein
10 or transcript be totally unique, in the sense that the particular INM marker molecule is present in the target cell type and in no other. Rather, it is required that the marker molecule have a signal to noise ratio high enough to discriminate the preselected cell type in
15 samples for which the assay is designed. For example, MT1 and MT2 proteins are useful as proteins indicating the presence of malignancy in cell samples because of their elevated expression levels in malignant cells, even though the proteins, or close analogs thereof, may
20 be present commonly in nonmalignant cell types.

A brief description of general protein and nucleic acid assay considerations follows below. Details of particular assay conditions may be found in the assay
25 references described above and incorporated herein by reference, and in published protocols well known in the art and readily available.

A. Protein Assays

30

Characterization of the MT1 and MT2 proteins at the molecular level as described herein allows one to characterize the proteins structurally and biochemically. Accordingly, following the disclosure
35 of these genetic sequences and their encoded amino acid

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sequences, preferred binding epitopes may be identified which may be used to enhance assay conditions. For example, binding proteins may be designed which have enhanced affinity for the marker protein produced by particular cell types or as a function of particular malignancies. Similarly, binding proteins may be designed which bind preferentially to protein fragments released from dying cells. In addition, structural and/or sequence variations between proteins produced in normal and abnormal tissue now may be investigated and used to advantage. The genetic sequences may be manipulated as desired, e.g., truncated, mutagenized or the like, using standard recombinant DNA procedures known in the art, to obtain proteins having desired features useful for antibody production.

As will be appreciated by those skilled in the art, any means for specifically identifying and quantifying a marker protein of interest is contemplated. The currently preferred means for detecting a protein of interest in a sample is by means of a binding protein capable of interacting specifically with the marker protein. Labelled antibodies or the binding portions thereof in particular may be used to advantage. The antibodies may be monoclonal or polyclonal in origin, or may be biosynthetically produced. The amount of complexed marker protein, e.g., the amount of marker protein associated with the binding protein, then is determined using standard protein detection methodologies well described in the art.

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A.1. Immunoassays

A variety of different forms of immunoassays currently exist, all of which may be adapted to detect and quantitate INM proteins and protein fragments. For exfoliated cell samples, as an example, the cells and surrounding fluid are collected and the INM proteins selectively isolated by the method of Penman and Fey, described in U.S. Pat. Nos. 4,882,268 and 4,885,236. These proteins then preferably are separated by two-dimensional gel electrophoresis and the presence of the marker protein detected by standard Western blot procedures.

For serum and other fluid assays where the marker proteins and/or protein fragments to be detected exist primarily in solution, one of the currently most sensitive immunoassay formats is the sandwich technique. In this method, as described in PCT publication WO93/09437 and which has a precision typically of $\pm 5\%$, two antibodies capable of binding the analyte of interest generally are used: e.g., one immobilized onto a solid support, and one free in solution, but labeled with some easily detectable chemical compound. Examples of chemical labels that may be used for the second antibody include radioisotopes, fluorescent compounds, and enzymes or other molecules which generate colored or electrochemically active products when exposed to a reactant or enzyme substrate. When samples containing the marker protein or protein fragment are placed in this system, the marker protein binds to both the immobilized antibody and the labelled antibody. The result is a "sandwich" immune complex on the support's surface. The complexed protein is detected by washing

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away nonbound sample components and excess labeled antibody, and measuring the amount of labeled antibody complexed to protein on the support's surface. The sandwich immunoassay is highly specific and very sensitive, provided that labels with good limits of detection are used. A detailed review of immunological assay design, theory and protocols can be found in numerous texts in the art, including Practical Immunology, Butt, W.R., ed., Marcel Dekker, New York, 1984.

In general, immunoassay design considerations include preparation of antibodies (e.g., monoclonal or polyclonal) having sufficiently high binding specificity for their antigen that the specifically-bound antibody-antigen complex can be distinguished reliably from nonspecific interactions. As used herein, "antibody" is understood to include other binding proteins having appropriate binding affinity and specificity for the marker protein. The higher the antibody binding specificity, the lower the antigen concentration that can be detected. Currently preferred binding specificity is such that the binding protein has a binding affinity for the marker protein of greater than about 10^5 M^{-1} , preferably greater than about 10^7 M^{-1} .

Antibody binding domains also may be produced biosynthetically and the amino acid sequence of the binding domain manipulated to enhance binding affinity with a preferred epitope. Identification of the genetic sequences for MT1 and MT2 can be used to advantage in the design and construction of preferred binding proteins. For example, a DNA encoding a preferred epitope may be recombinantly expressed and

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used to select an antibody which binds selectively to the eptiope. The selected antibodies then are exposed to the sample under conditions sufficient to allow specific binding of the antibody to its specific

5 nuclear matrix protein or protein fragment, and the amount of complex formed then detected. Specific antibody methodologies are well understood and described in the literature. A more detailed description of their preparation can be found, for

10 example, in Practical Immunology, Butt, W.R., ed., Marcel Dekker, New York, 1984.

The choice of tagging label also will depend on the detection limitations desired. Enzyme assays (ELISAs)

15 typically allow detection of a colored product formed by interaction of the enzyme-tagged complex with an enzyme substrate. Alternative labels include radioactive or fluorescent labels. The most sensitive label known to date is a chemiluminescent tag where

20 interaction with a reactant results in the production of light. Useful labels include chemiluminescent molecules such as acridium esters or chemiluminescent enzymes where the reactant is an enzyme substrate. When, for example, acridium esters are reacted with an

25 alkaline peroxide solution, an intense flash of light is emitted, allowing the limit of detection to be increased 100 to 10,000 times over those provided by other labels. In addition, the reaction is rapid. A detailed review of chemiluminescence and immunoassays

30 can be found in Weeks, et al., (1983) Methods in Enzymology 133:366-387. Other considerations for fluid assays include the use of microtiter wells or column

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immunoassays. Column assays may be particularly advantageous where rapidly reacting labels, such as chemiluminescent labels, are used. The tagged complex can be eluted to a post-column detector which also
5 contains the reactant or enzyme substrate, allowing the subsequent product formed to be detected immediately.

A.2. Antibody Production

10 The proteins described herein may be used to raise antibodies using standard immunological procedures well known and described in the art. See, for example, Practical Immunology, Butt, N.R., ed., Marchel Dekker, NY, 1984. Briefly, an isolated INM protein produced,
15 for example, by recombinant DNA expression in a host cell, is used to raise antibodies in a xenogenic host. Preferred antibodies are antibodies that bind specifically to an epitope on the protein, preferably having a binding affinity greater than 10^5M^{-1} , most
20 preferably having an affinity greater than 10^7M^{-1} for that epitope. For example, where antibodies to a human INM protein, e.g. MT1 or MT2 is desired, a suitable antibody generating host is a mouse, goat, rabbit, guinea pig, or other mammal useful for generating
25 antibodies. The protein is combined with a suitable adjuvant capable of enhancing antibody production in the host, and injected into the host, for example, by intraperitoneal administration. Any adjuvant suitable for stimulating the host's immune response may be used
30 to advantage. A currently preferred adjuvant is

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Freund's complete adjuvant (an emulsion comprising killed and dried microbial cells, e.g., from Calbiochem Corp., San Diego, or Gibco, Grand Island, NY). Where multiple antigen injections are desired, the subsequent
5 injections comprise the antigen in combination with an incomplete adjuvant (e.g. cell-free emulsion).

Polyclonal antibodies may be isolated from the antibody-producing host by extracting serum containing
10 antibodies to the protein of interest. Monoclonal antibodies may be produced by isolating host cells that produce the desired antibody, fusing these cells with myeloma cells using standard procedures known in the immunology art, and screening for hybrid cells
15 (hybridomas) that react specifically with the INM protein and have the desired binding affinity.

Provided below is an exemplary protocol for monoclonal antibody production, which is currently
20 preferred. Other protocols also are envisioned. Accordingly, the particular method of producing antibodies with the cancer marker protein compositions of this invention, is not envisioned to be an aspect of the invention. Also described below are exemplary
25 sandwich immunoassays and dot blot assays useful for detecting and/or quantitating marker proteins in a sample. Other means for detecting marker proteins, particularly MT1, MT2 and their analogs, including protein fragments and naturally-occurring variants,
30 also are envisioned. These other methods are well-known and described in the art.

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Exemplary antibody production protocol: Balb/c by J mice (Jackson Laboratory, Bar Harbor, ME) are injected intraperitoneally with purified INM protein (e.g., MT1) purified from the human cervical cell line CaSki, every 2 weeks for a total of 16 weeks. The mice are injected with a single boost 4 days prior to sacrifice and removal of the spleen. Freund's complete adjuvant (Gibco, Grand Island) is used in the first injection, incomplete Freund's in the second injection; subsequent injections are made with saline. Spleen cells (or lymph node cells) then are fused with a mouse myeloma line, e.g., using the method of Kohler and Milstein (1975) Nature 256:495, the disclosure of which is incorporated herein by reference, and using polyethylene glycol (PEG, Boehringer Mannheim, Germany). Hybridomas producing antibodies that react with nuclear matrix proteins then are cloned and grown as ascites. Hybridomas are screened by nuclear reactivity against the cell line that is the source of the immunogen, and by tissue immunochemistry using standard procedures known in the immunology art. Detailed descriptions of screening protocols, ascites production and immunoassays also are disclosed in PCT publication WO93/09437.

25

Exemplary Assays:

A. Sandwich Immunoassay (ELISA)

30 A standard immunoassay can be performed to generate dose response curves for antigen binding, for cross reactivity assays, and for monitoring assays. The data

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is generated with a standard preparation of NM antigen, and is used as the reference standard when body fluids are assayed. In these examples both ELISAs and radioummunoassays were performed.

5

1. Immunoassay (Well Assay)

Microtitre plates (Immulon II, Dynatech, Chantilly, VA) are coated with purified antibody at 5 to 15ug/ml in PBS at pH 7.4 for 1hr or overnight and then washed 3
10 x with 300 μ l PBS. The plates then are blocked with 10% normal goat serum in PBS for 1hr at room temp and washed 3 x with 300 μ l of PBS. An exemplary protocol follows.

15

Here, samples are assayed by pipetting 100 μ l of sample per well, and incubating for 1hr at RT. The wells were washed with 3 x 300 μ l PBS. 100 μ l of 1.25 to 10 μ g/ml of a biotinylated antibody added to each well,
20 incubated for 1hr at RT and washed with 3 x 300 μ l of PBS. 100 μ l of a 1:1000 dilution of streptavidin-horseradish peroxidase conjugate (The Binding Site Ltd., Birmingham, UK) added to each well and incubated for 1hr and then washed with PBS. 100 μ l of peroxidase
25 substrate (citrate, phosphate, OPD-H₂O₂) then is added to each well and incubated for 20min. The reaction is stopped by adding 50 μ l of 1M H₂SO₄ to the wells. The optical density is read on a plate reader at 490nm.

30

Concentrations of antigen are determined by preparing a reference concentration of antigen and preparing a standard dilution curve to compare with the unknown samples.

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2. IRMA (Immunoradiometric Assay)

(a) Iodination of Streptavidin.

10µg of streptavidin (Sigma, Inc., Cincinnati) in
5 2µl of 0.05M phosphate pH 7.4 was added to 10µl of
0.25M phosphate pH 7.4 in a microcentrifuge tube and
1mCi of ¹²⁵I (NEN-DUPONT, Wilmington, DE) in 10µl is
added. Immediately 10µl of 100mg chloramine-T
trihydrate (Sigma, Inc.) in 50ml of distilled water is
10 added, mixed, and reacted for 25sec. The reaction then
is stopped by mixing for 20sec with 50µl of 40mg
Cysteamine (2-mercaptoethylamine)(Sigma, Inc.) and 5mg
KI in 50ml of 0.05M phosphate pH 7.4. 0.5ml of 1% BSA
in PBS pH 7.4 added and the material fractionated on a
15 10ml sephadex G-100 column (Pharmacia, Sweden) pre-
equilibrated with the BSA PBS buffer. 30 by 0.5ml
fractions are collected and 10µl diluted to 1ml of the
BSA/PBS buffer for each fraction. 100µl of the diluted
fraction is counted on a LKB gamma counter set for
20 ¹²⁵I. The specific activity is calculated and
routinely falls between 85 to 100uCi/ug. The mid
fractions of the protein peak then are used in the
sandwich immunoassay.

25 (b) Sandwich Radioimmunoassay.

The microtitre breakaway wells (Immulon II
Removawell strips, Dynatech, Chantilly, Va) are coated
and blocked as in the ELISA assay. The samples,
30 standard or sera, are routinely measured by incubating
100µl in the wells for 1hr at RT washing on a plate
washer with 3 x 300µl of PBS and then incubated with
the biotinylated antibody (2-10µg/ml in 10% goat serum)
for 1hr at RT and washed again. The bound biotinylated
35 antibody is detected with the ¹²⁵I-streptavidin.

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200,000 to 300,000 cpm (77% counter efficiency) in 100 μ l is added to each well and incubated for 1hr at RT and washed again. The bound fraction is detected by counting the radioactivity in an LKB gamma counter.

- 5 The concentration can be determined by comparing the counts obtained against a reference preparation.

B. Dot Blot Detection of NM.

- 10 Antibody reactivity with NM proteins can be assessed by dot blot detection assays, using standard methodologies and apparatus (e.g., Schleicher & Schuell). Nitrocellulose membranes are soaked in Tris buffered saline, (TBS, 50mM TRIS, 150mM NaCl, pH 7.6)
- 15 and NM preparation applied at varying concentrations of protein to a series of wells and incubated for 1hr at room temperature (e.g., T-47D NM supernatant at 10 μ g/ml, 1 μ g/ml and 100ng/ml). The blocked wells then are washed with 2 x 200 μ l of TBS and then blocked with
- 20 100 μ l 10% normal goat serum in TBS for 1hr at room temperature. The blocked wells then are washed again with 2 x 200 μ l of TBS and 100 μ l of culture supernatant containing nuclear reactive antibody to be tested is added to their respective wells and incubated for 1hr
- 25 at room temperature. The wells then are washed with 2 x 200 μ l of TBS and 100 μ l of a dilution series of alkaline phosphatase conjugated goat anti-mouse IgG (Bio-Rad, Richmond, CA) (e.g., 1:1000, 1: 5000, or 1:10000) added to the relevant wells and incubated for
- 30 1hr. The wells then are washed with 2 x 200 μ l of TBS followed by addition of enzyme substrate (BCIP/NBT, Kirkgaard and Perry, Gaithersburg, MD, e.g., 100 μ l) in Tris buffer containing Levamisole (Vector, Inc., Corpus Christi, TX.) A fifteen minute incubation generally is
- 35 sufficient. The reaction can be stopped by washing with distilled water and the product detected.

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B. Nucleic Acid Assays

The status of a tissue also may be determined by detecting the quantity of transcripts encoding these cancer marker proteins. The currently preferred means for detecting mRNA is by means of northern blot analysis using labeled oligonucleotides e.g., nucleic acid fragments capable of hybridizing specifically with the transcript of interest. The currently preferred oligonucleotide sequence is a sequence encoding a complementary sequence to that of at least part of the transcript marker sequence. These complementary sequences are known in the art as "antisense" sequences. The oligonucleotides may be oligoribonucleotides or oligodeoxyribonucleotides. In addition, oligonucleotides may be natural oligomers composed of the biologically significant nucleotides, i.e., A (adenine), dA (deoxyadenine), G (guanine), dG (deoxyguanine), C (cytosine), dC (deoxycytosine), T (thymine) and U (uracil), or modified oligonucleotide species, substituting, for example, a methyl group or a sulfur atom for a phosphate oxygen in the inter-nucleotide phosphodiester linkage. (see, for example, Section I.C, below.) Additionally, the nucleotides themselves, and/or the ribose moieties may be modified.

The sequences may be synthesized chemically, using any of the known chemical oligonucleotide synthesis methods well described in the art. For example, the oligonucleotides are advantageously prepared by using any of the commercially available, automated nucleic acid synthesizers. Alternatively, the oligonucleotides may be created by standard recombinant DNA techniques, by, for example, inducing transcription of the noncoding strand. For example, the DNA sequence

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encoding a marker protein may be inverted in a recombinant DNA system, e.g., inserted in reverse orientation downstream of a suitable promoter, such that the noncoding strand now is transcribed.

5

Useful hybridizing oligonucleotide sequences include any sequences capable of hybridizing specifically to the MT1 or MT2 primary transcripts. Accordingly, as will be appreciated by those skilled in the art, useful sequences contemplated include both sequences complementary to the DNA sequences provided in Seq. ID No. 1 (MT1) or Seq. ID No. 3 (MT2) which correspond to the protein coding regions, as well as sequences complementary to transcript sequences occurring further upstream or downstream from the coding sequence (e.g., sequences contained in, or extending into, the 5'- and 3' untranslated regions). Representative antisense sequences are described in Seq. ID Nos. 5 and 6. Seq. ID No. 5 describes a sequence complementary to the first 100 nucleotides of the MT1 protein coding sequence (compare Seq. ID Nos. 1 and 5) as well as the 53 nucleotide sequence occurring upstream of the initiation codon. The complementary nucleotides to the initiation codon occur at positions 298-300 in Seq. ID No. 5. Similarly, Seq. ID No. 6 describes a sequence complementary to the first 100 nucleotides of the MT2 protein coding sequence (compare Seq. ID Nos. 3 and 6), as well as the 48 nucleotide sequence occurring upstream of the initiation codon. The complementary nucleotides to the initiation codon occur at positions 298-300 in Seq. ID No. 6. Useful oligomers may be created based on part or all of the sequences in Seq. ID No. 5 and 6. However, as will be appreciated by those skilled in the art, other useful sequences which hybridize to other

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regions of the transcript readily are created based on the sequences presented in Seq. ID Nos. 1 and 3 and/or additional, untranslated sequences, such as are disclosed for MT2 (NuMA) in Compton et al. and Yang et al.

While any length oligonucleotide may be utilized to hybridize an mRNA transcript, sequences less than 8-15 nucleotides may be less specific in hybridizing to target mRNA. Accordingly, oligonucleotides typically within the range of 8-100 nucleotides, preferably within the range of 15-50, nucleotides are envisioned to be most useful in standard RNA hybridization assays.

The oligonucleotide selected for hybridizing to the INM transcript, whether synthesized chemically or by recombinant DNA, then is isolated and purified using standard techniques and then preferably labelled (e.g., with ^{35}S or ^{32}P) using standard labelling protocols.

A sample containing the marker transcript of interest then is run on an electrophoresis gel, the dispersed nucleic acids transferred to a nitrocellulose filter and the labelled oligonucleotide exposed to the filter under suitable hybridizing conditions, e.g. 50% formamide, 5 X SSPE, 2 X Denhardt's solution, 0.1% SDS at 42°C, as described in Molecular Cloning: A Laboratory Manual, Maniatis et al. Other useful procedures known in the art include solution hybridization, and dot and slot RNA hybridization. The amount of marker transcript present in a sample then is quantitated by measuring the radioactivity of hybridized fragments, using standard procedures known in the art.

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Following a similar protocol, oligonucleotides also may be used to identify other sequences encoding members of the MT1 and MT2 protein families, for example, as described in the examples that follow. The methodology also may be used to identify genetic sequences associated with the protein coding sequences described herein, e.g., to identify noncoding sequences lying upstream or downstream of the protein coding sequence, and which may play a functional role in expression of these genes. Where new marker species are to be identified, degenerate sequences and/or sequences with preferred codon bias may be created, using the sequences of Seq. ID Nos. 1 or 3 as templates, and the general guidelines described in the art for incorporating degeneracies. (See, for example, Molecular Cloning: A Laboratory Manual, Maniatis, et al.)

C. Therapeutics

The proteins described herein are associated with the spindle apparatus during mitosis, and are present at elevated levels in malignant cells. Accordingly, without being limited to any particular theory, it is hypothesized that the proteins likely play a significant role in cell division, most likely a structurally related role. Accordingly, these proteins and their transcripts are good candidates as target molecules for a cancer chemotherapy.

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C.1 Antisense Therapeutics

A particularly useful cancer therapeutic envisioned is an oligonucleotide complementary to part all of the marker transcript, capable of hybridizing specifically to the transcript and inhibiting translation of the mRNA when hybridized to the mRNA transcript. Antisense oligonucleotides have been used extensively to inhibit gene expression in normal and abnormal cells. See, for example, Stein et al. (1988) Cancer Res. 48:2659-2668, for a pertinent review of antisense theory and established protocols. Accordingly, the antisense nucleotides to MT1 and MT2 may be used as part of chemotherapy, alone or in combination with other therapies.

As described in Section I.B above, both oligoribonucleotide and oligodeoxyribonucleotide sequences will hybridize to an mRNA transcript and may be used to inhibit mRNA translation of the marker protein described herein. However, oligoribonucleotides generally are more susceptible to enzymatic attack by ribonucleases than deoxyribonucleotides. Hence, oligodeoxyribonucleotides are preferred for in vivo therapeutic use to inhibit mRNA translation in an individual.

Also, as described in Section I.B above, the therapeutically useful antisense oligonucleotides of the invention may be synthesized by any of the known chemical oligonucleotide synthesis methods well described in the art. Alternatively, a complementary sequence to part or all of the natural mRNA sequence

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may be generated using standard recombinant DNA technology. For example, the DNA encoding the protein coding sequence may be inserted in reverse orientation downstream of a promoter capable of expressing the
5 sequence such that the noncoding strand is transcribed.

Since the complete nucleotide sequence of the protein coding sequence as well as additional 5' and 3' untranslated sequences are known for both MT1 and MT2
10 (see, for example, Seq. ID Nos. 1 and 3 and Compton et al.), and/or can be determined with this disclosure, antisense oligonucleotides hybridizable with any portion of the mRNA transcripts to these proteins may be prepared using conventional oligonucleotide
15 synthesis methods known to those skilled in the art.

Oligonucleotides complementary to and hybridizable with any portion of the MT1 and MT2 mRNA transcripts are, in principle, effective for inhibiting translation
20 of the transcript as described herein. For example, as described in U.S. Pat. No. 5,098,890, issued March 24, 1992, the disclosure of which is incorporated herein by reference, oligonucleotides complementary to mRNA at or near the translation initiation codon site may be used
25 to advantage to inhibit translation. Moreover, it has been suggested that sequences that are too distant in the 3' direction from the translation initiation site may be less effective in hybridizing the mRNA transcripts because of potential ribosomal "read-
30 through", a phenomenon whereby the ribosome is postulated to unravel the antisense/sense duplex to permit translation of the message.

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Representative antisense sequences for MT1 and MT2 transcripts are described in Seq. ID No. 5 (MT1) and Seq. ID No. 6 (MT2). The antisense sequences are complementary the sequence encoding the N-terminus of either the MT1 or MT2 marker protein, as well as part of the 5' untranslated sequences immediately upstream of the initiation codon. (See Section I.B, above for a detailed description of these sequences). As will be appreciated by those skilled in the art, antisense oligonucleotides complementary to other regions of the MT1 and/or MT2 transcripts are readily created using for example, the sequences presented in Seq. ID No. 1 and 3 as templates.

As described in Section I.B above, any length oligonucleotide may be utilized to hybridize to mRNA transcripts. However, very short sequences (e.g., less than 8-15 nucleotides) may bind with less specificity. Moreover, for in vivo use such short sequences may be particularly susceptible to enzymatic degradation. In addition, where oligonucleotides are to be provided directly to the cells, very long sequences may be less effective at inhibition because of decreased uptake by the target cell. Accordingly, where the oligonucleotide is to be provided directly to target cells, oligonucleotides having a length within the range of 8-50 nucleotides, preferably 15-30 nucleotides, are envisioned to be most advantageous.

An alternative means for providing antisense sequences to a target cell is as part of a gene therapy technique, e.g., as a DNA sequence, preferably part of a vector, and associated with a promoter capable of expressing the antisense sequence, preferably constitutively, inside the target cell. Recently,

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Oeller et al. ((1992) Science 254:437-539, the disclosure of which is incorporated by reference) described the in vivo inhibition of the ACC synthase enzyme using a constitutively expressible DNA sequence
5 encoding an antisense sequence to the full length ACC synthase transcript. Accordingly, where the antisense sequences are provided to a target cell indirectly, e.g., as part of an expressable gene sequence to be expressed within the cell, longer oligonucleotide
10 sequences, including sequences complementary to substantially all the protein coding sequence, may be used to advantage.

Finally, also as described in Section I.B, above,
15 the therapeutically usefully oligonucleotides envisioned include not only native oligomers composed of naturally occurring nucleotides, but also those comprising modified nucleotides to, for example, improve stability and lipid solubility and thereby
20 enhance cellular uptake. For example, it is known that enhanced lipid solubility and/or resistance to nuclease digestion results by substituting a methyl group or sulfur atom for a phosphate oxygen in the internucleotide phosphodiester linkage.
25 Phosphorothioates ("S-oligonucleotides" wherein a phosphate oxygen is replaced by a sulfur atom), in particular, are stable to nuclease cleavage, are soluble in lipids, and are preferred, particularly for direct oligonucleotide administration.
30 S-oligonucleotides may be synthesized chemically by the known automated synthesis methods described in Section I.B, above.

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Suitable oligonucleotide sequences for mRNA translation inhibition are readily evaluated by a standard in vitro assay using standard procedures described herein and well characterized in the art. An exemplary protocol is described below, but others are envisioned and may be used to advantage.

A candidate antisense sequence is prepared as provided herein, using standard chemical techniques. For example, an MT1 antisense sequence may be prepared having the sequence described by positions 285-315 of Sequence ID No. 5 using an Applied Biosystems automated DNA Synthesizer, and the oligonucleotide purified accordingly to manufacturer's instructions. The oligonucleotide then is provided to a suitable malignant cell line in culture, e.g., ME-180, under standard culture conditions, to be taken up by the proliferating cells.

Preferably, a range of doses is used to determine effective concentrations for inhibition as well as specificity of hybridization. For example, a dose range of 0-100 μ g oligonucleotide/ml may be assayed. Further, the oligonucleotides may be provided to the cells in a single transfection, or as part of a series of transfections.

Antisense efficacy may be determined by assaying a change in cell proliferation over time following transfection, using standard cell counting methodology and/or by assaying for reduced expression of marker protein, e.g., by immunofluorescence, as described in Section I.A, above. Alternatively, the ability of

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cells to take up and use thymidine is another standard means of assaying for cell division and maybe used here, e.g., using ³H thymidine. Effective antisense inhibition should inhibit cell division sufficiently to

5 reduce thymidine uptake, inhibit cell proliferation, and/or reduce detectable levels of marker proteins.

Useful concentration ranged are envisioned to vary according to the nature and extent of the neoplasm, the

10 particular oligonucleotide utilized, the relative sensitivity of the neoplasm to the oligonucleotides, and other factors. Useful ranges for a given cell type and oligonucleotide may be determined by performing a standard dose range experiment as described here. Dose

15 range experiments also may be performed to assess toxicity levels for normal and malignant cells. Concentrations from about 1 to 100 µg/ml per 10⁵ cells may be employed to advantage.

20 For in vivo use, the antisense oligonucleotides may be combined with a pharmaceutical carrier, such as a suitable liquid vehicle or excipient and an optional auxiliary additive or additives. The liquid vehicles and excipients are conventional and commercially

25 available. Illustrative thereof are distilled water, physiological saline, aqueous solutions of dextrose, and the like. For in vivo cancer therapies, the antisense sequences preferably are provided directly to the malignant cells, as by injection to the neoplasm

30 locus. Alternatively, the oligonucleotide may be administered systemically, provided that the antisense sequence is associated with means for directing the sequences to the target malignant cells.

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In addition to administration with conventional carriers, the antisense oligonucleotides may be administered by a variety of specialized oligonucleotide delivery techniques. For example, 5 oligonucleotides maybe encapsulated in liposomes, as described in Maniatis et al., Mannino et al. (1988) BioTechnology 6:682, and Felgner et al. (1989) Bethesda Res. Lab. Focus 11:21. Reconstituted virus envelopes also have been successfully used to deliver RNA and DNA 10 to cells. (see, for example, Arad et. al., (1986) Biochem. Biophy. Acta. 859, 88-94.)

For therapeutic use in vivo, the antisense oligonucleotides are provided in a therapeutically 15 effective amount, e.g., an amount sufficient to inhibit target protein expression in malignant cells. The actual dosage administered may take into account whether the nature of the treatment is prophylactic or therapeutic in nature, the age, weight, health and sex 20 of the patient, the route of administration, the size and nature of the malignancy, as well as other factors. The daily dosage may range from about 0.01 to 1,000 mg per day. Greater or lesser amounts of oligonucleotide may be administered, as required. As will be 25 appreciated by those skilled in the medical art, particularly the chemotherapeutic art, appropriate dose ranges for in vivo administration would be routine experimentation for a clinician. As a preliminary guideline, effective concentrations for in vitro 30 inhibition of the target molecule may be determined first, as described above.

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II.B PROTEIN INHIBITION

In another embodiment, the cancer marker protein itself may be used as a target molecule. For example, a binding protein designed to bind the marker protein essentially irreversibly can be provided to the malignant cells e.g., by association with a ligand specific for the cell and known to be absorbed by the cell. Means for targeting molecules to particular cells and cell types are well described in the chemotherapeutic art.

Binding proteins maybe obtained and tested as described in Section I.A above. For example, the binding portions of antibodies maybe used to advantage. Particularly useful are binding proteins identified with high affinity for the target protein, e.g., greater than about 10^9 M^{-1} . Alternatively, the DNA encoding the binding protein may be provided to the target cell as part of an expressable gene to be expressed within the cell following the procedures used for gene therapy protocols well described in the art. (see, for example, U.S. Patent No. 4,497,796, and Gene Transfer, Vijay R. Baichwal, ed., (1986). It is anticipated that the complexed INM protein will be disabled and can inhibit cell division thereby.

As described above for antisense nucleotides, for in vivo use, suitable binding proteins may be combined with a suitable pharmaceutical carrier, such as physiological saline or other useful carriers well characterized in the medical art. The pharmaceutical compositions may be provided directly to malignant cells, e.g., by direct injection, or may be provided systemically, provided the binding protein is

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associated with means for targeting the protein to target cells. Finally, suitable dose ranges and cell toxicity levels may be assessed using standard dose range experiments. Therapeutically effective
5 concentrations may range from 0.1-1,000 mg per day. As described above, actual dosages administered may vary depending, for example, on the nature of the malignancy, the age, weight and health of the individual, as well as other factors.

10

II. EXEMPLIFICATION

The following examples further describe the utility of MT1 and MT2 as markers for abnormal cell types, and
15 how the genetic sequences encoding MT1 and MT2 proteins were isolated and characterized, including the current best mode for their cloning and characterization, without limiting the scope thereof. For example, INM protein expression in E. coli is described herein.
20 However, other prokaryotic and eukaryotic cell expression systems also are contemplated for recombinant expression of the proteins described herein. Other useful hosts contemplated include Saccharomyces, the insect/baculovirus expression
25 system, and mammalian cells such as xenogenic myeloma cells and the well-characterized chinese hamster ovary cell lines.

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MT1

As demonstrated below, MT1 expression levels are enhanced significantly in a number of different malignant cell types, including malignant breast, colon, bladder, ovary, prostate and cervix cell types. Presented below are the results of a standard immunoassay (precision $\pm 5\%$), performed as described herein and in PCT publication WO93/03497 on nuclear matrix (NM) preparations made from normal and malignant human tissue extracts and which were prepared essentially as described therein (in 8M urea, 2% β -mercaptoethanol, 2% Nonidet P-40 (detergent).) The 302.47 antibody was raised against a NM preparation from CaSki, a cultured cervical tumor cell line (American Type Culture Collection, ATCC, Rockville, MD). MT1:2-8 was raised against the cloned MT1 protein. Both antibodies bind to epitopes on the protein encoded by Seq. ID No.1, as demonstrated using standard binding assays. As can be seen from the results presented below, MT1 is significantly elevated in malignant bladder tissue. Blotting experiments also indicate MT1 levels are elevated in other malignant tissues.

TABLE I

25

<u>Sample</u>	<u>Antibody Combination</u>	<u>ng MT-1/ g tissue</u>
normal bladder	302.47/MT1:2-8	13,500
bladder cancer	302.47/MT1:2-8	32,000

30

Cloning

The natural-sourced MT1 protein first was separated from human cervical tumor cells essentially following the procedure of Penman and Fey described in U.S. Pat. Nos. 4,882,268 and 4,885,236. Cells from the human

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cervical tumor cell lines CaSki and ME180 (obtained from the American Tissue Culture Collection, ATCC, Rockville, MD) were grown to confluence and removed from flasks by trypsinization. Suspended cells were

5 washed twice with phosphate buffered saline (PBS) and extracted with cytoskeletal buffer (CSK): 100 mM NaCl, 300 mM sucrose, 10 mM PIPES, 3 mM MgCl₂, 1 mM EGTA, 0.5% Triton X-100, 1.2 mM PMSF for 1 min at 4°C, followed by extraction in cold RSB (reticulocyte

10 suspension buffer)/double detergent buffer: 100 mM NaCl, 3 mM MgCl₂, 10 mM Tris, pH 7.4, 1% Tween 40, 0.5% deoxycholate, 1.2 mM PMSF. Alternatively, cells were extracted twice with the RBS/double detergent buffer. The two extraction protocols produced very similar

15 preparations. The extracted cells were digested for 30 min at room temperature in digestion buffer: 50mM NaCl, 300 mM sucrose, 0.5% Triton X-100, 10 mM PIPES (pH 6.8), 3 mM MgCl₂, 1mM EGTA, 1.2 mM PMSF, containing 100 µg of both RNase A and DNase I. Chromatin was

20 extracted from the digested nuclei by the addition of 2 M ammonium sulfate to a final concentration of 0.25 M. The extracted nuclear matrix-intermediate filament (NM-IF) scaffolds then were sedimented at 3700 x g for 15 min.

25

The resulting pellet then was resuspended in disassembly buffer: 8 M urea, 20 mM MES (pH 6.6), 1 mM EGTA, 1.2mM PMSF, 0.1 mM MgCl₂, 1% 2-mercaptoethanol, and the pellet sonicated and dialyzed overnight with

30 3 changes of 2000 volumes of assembly buffer: 0.15 M KCl, 25 mM imidazole (pH 7.1), 5 mM MgCl₂, 2 mM DTT, 0.125 mM EGTA, 0.2 mM PMSF. The dialysate then was centrifuged at 100k x g for 1 h and the NM proteins recovered from the supernatant. Alternatively, NM-IF

35 scaffolds were extracted directly with E400 buffer:

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0.4 M NaCl, 0.02 M Tris pH 7.5, 0.1 mM MCl_2 , 0.5% 2-mercaptoethanol, 1.2 mM PMSF, for 30 min at 4°C, as described by von Kries et al. (1991) Cell 64:123-135. The intermediate filament-rich pellet then was removed
5 after centrifugation for 90 min at 40K rpm in a Beckman 70.1 Ti rotor. The supernatant remaining is enriched in MT1 protein with little cytokeratin contamination.

MT1-specific antibodies were produced by standard
10 procedures. Specifically, Balb/c by J mice (Jackson Laboratory, Bar Harbor, ME) were injected intraperitoneally with purified Caski NM protein every 2 weeks for a total of 16 weeks. The mice were injected with a single boost 4 days prior to sacrifice
15 and removal of the spleen. Freund's complete adjuvant was used in the first injection, incomplete Freund's in the second injection; subsequent injections were made with saline. Spleen cells were fused with the SP2/O-Ag14 mouse myeloma line (ATCC, Rockville, MD) using the
20 standard fusion methodologies well known in the art. Hybridomas producing antibodies that reacted with nuclear matrix proteins were cloned and grown as ascites. Antigen specificity was assessed both by immunofluorescence spectroscopy and Western blot
25 analysis. The 302.47 antibody was used to screen an expression library as described below to isolate the MT1 gene.

The cDNA clones for MT1 were obtained from a Lambda
30 ZAP expression library (Stratagene, La Jolla, CA). Library screening was carried out according to the manufacturer's instructions and using the MT1-specific antibody 302.47. Briefly, a single positive clone

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containing a 2.45 kb insert was identified and subcloned into pBluescript II vectors (Stratagene, La Jolla, CA) opened at the EcoRI and XhoI cloning sites. The resulting plasmid, pMT1, was sequenced
5 directly and further subcloned to produce the MT1 fusion protein (see below).

The cDNA sequences were obtained using the standard dideoxy method described in the art. Double stranded
10 sequencing was done utilizing the pMT1 vector primed with appropriate primers according to manufacturer's instructions (Stratagene, La Jolla, CA). Internal sequences were obtained using synthetic primers, created based on the identified sequence.

15 The entire nucleotide sequence and predicted amino acid sequence for MT1 are shown in Seq. ID No. 1. The cDNA clone retains a polyadenylation signal a putative initiation codon, a continuous open reading frame and
20 codon utilization consistent with a human gene. The predicted amino acid sequence of MT1 consists of 639 amino acids encoding a protein of 70.5 kD with a pI of 5.47. The primary structure, as predicted by the Chou-Fasman algorithm (Chou and Fasman, (1978) Adv. Enzymol. Relat. Areas Mol. Biol. 47:145-148), consists of 72%
25 alpha helix of which 56% is extended helix.

The primary structure of MT1, represented in Fig. 1, contains 27 proline residues which generally
30 occur in pairs or triplets throughout the molecule. The proline distribution within the sequence is illustrated in Fig. 1A, where diamonds represent the proline residues. Proline pairs and triplets are indicated by stacked diamonds. At the N terminus, a
35 40 amino acid stretch contains a cluster of 8 prolines

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(residues 42-81, Seq. ID No. 1) that occur as pairs separated by 3 or fewer amino acids. A similar proline-rich region occurs in the C terminus of MT1 (residues 551-563) where 6 prolines occur in a 13 amino acid stretch. Both proline-rich regions likely lie on the protein surface, based on probability calculations determined by the technique of Emini et al. (1985) J. Virol. 55:836-839. The high proline density also may explain the anomalous apparent molecular weight of the protein as determined by SDS polyacrylamide gel electrophoresis. As described above, the predicted molecular weight for MT1, calculated from the amino acid sequence, is 70.1 kD. However, as described below, both the natural-sourced and recombinant protein migrate as a 90 kD protein on an SDS polyacrylamide gel. Alternatively, it is also possible that the molecular weight variation may result from some post-translational modification achievable in both prokaryotic and eukaryotic cells.

Between the two proline-rich termini, MT1 displays a sequence consistent with a region of extended alpha helix structure, indicated by the hatched structure in Fig. 1B. The extended helix is interrupted in 4 places by short helix-distorting amino acid stretches that usually include a pair of proline residues. A preliminary hypothesis as to the structure of MT1 based on these theoretical calculations is that the molecule consists of an extended rod that is bounded on either end by a globular, proline-rich domain.

Analysis of all available sequence databases indicates that MT1 has a novel sequence that bears no significant homology to any known protein. In

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addition, the sequence appears to lack any known, identifiable DNA binding motif such as the leucine zipper motif.

5 The cloned MT1 DNA was used to perform standard Northern blot analysis of total and poly A+ RNA from ME180 cells, using standard procedures and 15 µg RNA. After blotting and hybridization with ³²P-labelled pMT1 DNA, a single mRNA band was detected in the poly A+
10 fraction. This band was not apparent in the total RNA lane after a 48 h exposure of the autoradiogram, indicating that the MT1 message is a low abundance species. Northern blot analysis indicates that the MT1 protein is translated from a single mRNA. Northern
15 blot analysis also indicates that the MT1 RNA includes approximately 500 bp 5' of the protein-coding sequence presented in Seq. ID No. 1. This upstream sequence may represent one or more untranslated sequences and/or may encode additional protein coding sequences.

20

A fusion protein for MT1 was obtained using the insert from the pMT1 construct described above and in Seq. ID No. 1, and the pMAL expression system (New England Biolabs Inc., Beverly, MA). In this system the
25 gene of interest (MT1) is cloned into the pMal-c vector (New England Biolabs Inc., Beverly, MA) and the vector transfected into E. coli and expressed to produce a fusion protein containing both the protein of interest and the maltose binding protein. The maltose binding
30 protein allows the fusion protein to be selectively purified in the presence of maltose and can be subsequently cleaved by proteolytic cleavage with Factor Xa to yield intact, recombinant MT1 protein. Here, MT1 cDNA was cloned into the pMAL-c vector such that the
35 initiation AUG codon was directly continuous with the

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5' terminus of the maltose binding protein. After proteolytic cleavage with factor Xa the resulting MT1 fusion protein retains the complete amino acid sequence encoded by the MT1 cDNA with no additional amino acids.

- 5 All experimental details of the pMAL system were carried out according to the manufacturer's instructions.

- As described above, both the natural-sourced and recombinaantly produced protein have an electrophoretic mobility consistent with an apparent molecular weight of about 90kD on SDS-PAGE. In addition, the pI of both proteins is equivalent (5.4) and consistent with the predicted pI as calculated from the amino acid sequence. Peptide mapping of both proteins by cleavage at cysteine residues with 2-nitro-5-thiocyanobenzoic acid (NTCB), following the method of Leto and Marchesi (1984) J. Biol. Chem. 259:4603-4049, yields equivalent peptide fragments which share the same MT1 cross reactivity by Western blot analysis. Moreover, the number and size of the peptide fragments produced are consistent with those predicted from the proposed MT1 amino acid sequence.

25

MT2

- Like MT1, MT2 expression levels are enhanced significantly in a variety of malignant cell types, as determined both by serum assays and tissue culture supernatant assays. In the assays described below, the antibodies used were raised against two different cervical tumor cell line NM preparations (ME-180 and CaSKi, ATTC, Rockville, MD.) The 100-series antibodies are those raised against the ME-180 immunogen; the 300-

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series antibodies are those raised against CaSKi-NM immunogen. Of the antibodies described below, 107.7 and 307.33 have been determined to bind specifically with the MT2 protein, and 302-18, 302-22 and 302-29 cross react with a protein closely associated with MT2 and which co-isolates with it.

Dose response evaluation results of two antibody combinations are shown in Table II, below, using ME-180 cell culture supernatant as the antigen source. Each assay shows dose dependent detection of antigen in the tissue culture supernatant, demonstrating the ability of the assay to quantitate soluble interior nuclear matrix protein released from dying cells.

Table II

Antibody 107-7 solid phase, 302-29 soluble antibody, ME-180 supernatant

<u>Concentration of supernatant</u>	<u>Mean OD</u>	<u>SD</u>
2:1	0.501	0.013
undiluted	0.274	0.018
1:2	0.127	0.006
1:4	0.067	0.006
1:8	0.035	0.009
1:16	0.021	0.007
No Sup	0.000	

Antibody 107-7 solid phase, 307-33 soluble antibody, ME-180 supernatant.

<u>Concentration of supernatant</u>	<u>Mean OD</u>	<u>SD</u>
3:1	0.906	0.009
3:2	0.456	0.011
3:4	0.216	0.007
3:8	0.099	0.005
3:16	0.052	0.002
3:32	0.031	0.005
No Sup		

45

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Next, interior nuclear matrix protein quantification was tested in supernatant from a variety of dying tumor tissues. Here, tumor and normal tissues were allowed to die in media by serum deprivation.

5 Specifically, cell lines were grown to confluency in tissue culture flasks by standard culturing techniques. The media then was replaced with serum-free media and the cells placed in a 37°C incubator with 5% CO₂ for 7 to 14 days. At the end of the incubation the media was

10 collected and centrifuged at 14,000xg to remove cellular debris. Supernatants were assayed in various configurations of sandwich assays.

The results are shown in Fig. 3, where all values

15 are in units/gm, using ME-180 antigen as standard. As can be seen from Fig. 3, MT2 antigen is released from each of the dying tissues and the increased cell death in tumor tissue is reflected in a higher MT2 average antigen value quantitated in cancer tissue versus

20 normal tissue.

Figure 4 shows the results of an analogous experiment performed using serum samples from cancer patients and normal blood donors. Here tissue is

25 prepared as follows. Tissue is removed from a donor, flash frozen in liquid nitrogen within 10min to 4hrs after removal and stored at -70°C until required. When ready to be used, the tissue is chopped into 0.1 to 0.3 cm cubes as it thaws using aseptic techniques in a

30 laminar flow hood and placed in a T150 flask containing serum free media containing Fungizone and gentamycin. In general, 2-4g of tissue are used per 100ml media in the T150 flask. The flask containing the tissue then is incubated for 4-7 days at 37°C with 5% CO₂. After

35 incubation the media is collected from the flasks,

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centrifuged at 14,000xg for 20min. As for Fig. 3, ME-180 cell antigen was the standard. Results are reported in units/ml. A control experiment diluting supernatant antigen into serum and then quantitating the protein in solution indicates that serum has little or no effect on the assay. As can be seen in the results presented in Fig. 4, like the results shown in Fig. 3, serum samples from cancer patients reflect a higher rate of cell death as indicated by the quantifiably higher levels of MT2 antigen detected in these samples compared with those detected in the normal blood serum samples.

Cloning

Following the same general procedure as for MT1, a composition selectively enriched for MT2 was obtained from ME-180 cells (cervical carcinoma cells, from ATCC, Rockville, MD), and MT2-specific antibodies prepared. The 107.7 antibody was used to obtain a partial cDNA clone for MT2, by screening a lambda ZAP expression library, as for MT-1. The partial clone retrieved then was subcloned into a pBluescript II vector (pMT2) and the MT2 cDNA sequenced using standard techniques. The sequenced DNA, which corresponds to residues 1366 to 2865 of Seq. ID No. 3, then was analyzed to determine the reading frame and encoded amino acid sequence. The complete coding sequence subsequently was determined and is presented in Seq. ID No. 3. (Compton et al. (1992) J. Cell Biol. 116: 1395-1408). The nucleotide sequence and predicted amino acid sequence for MT2 are described in Seq. ID No. 3.

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The primary structure of MT2 is represented schematically in Fig. 2: The protein appears to comprise at least 6 helical regions separated by proline pairs, (See Fig. 4A and B.) The primary
5 structure may allow the protein to form a coiled-coil structure in solution. As for Fig. 3, prolines are indicated by diamonds and helices by hatched boxes. In addition, both the N and C termini of MT2 appear to fold as globular domains (Compton et al. (1992) J. Cell
10 Biol. 116: 1395-1408.)

The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The present embodiments are
15 therefore to be considered in all respects as illustrative and not restrictive, the scope of the invention being indicated by the appended claims rather than by the foregoing description, and all changes which come within the meaning and range of equivalency
20 of the claims are therefore intended to be embraced therein.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(1) APPLICANT:

- (A) NAME: Matritech, Inc.
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- (E) COUNTRY: USA
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- (G) TELEPHONE: 1-617-661-6660
- (H) TELEFAX: 1-617-661-8522
- (I) TELEX:

(11) TITLE OF INVENTION: NOVEL MALIGNANT CELL TYPE MARKERS
OF THE INTERIOR NUCLEAR MATRIX

(iii) NUMBER OF SEQUENCES: 6

(iv) CORRESPONDENCE ADDRESS:

- (A) ADDRESSEE: TESTA HURWITZ & THIBEAULT
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- (E) COUNTRY: USA
- (F) ZIP: 02109

(v) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

- (A) APPLICATION NUMBER: US
- (B) FILING DATE:
- (C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

- (A) NAME: PITCHER ESQ, EDMUND R
- (B) REGISTRATION NUMBER: 27,829
- (C) REFERENCE/DOCKET NUMBER: MTP-013

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- (A) TELEPHONE: 617/248-7000
- (B) TELEFAX: 617/248-7100

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2360 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: HOMO SAPIENS
- (F) TISSUE TYPE: CERVIX TUMOR

GAGATGGTTC TTGGTCCTGC AGCTTATAAT GTTCCATTGC CAAAGAAATC GATTCAGTCG	60
GGTCCACTAA AAATCTCTAG TGTATCAGAA GTA ATG AAA GAA TCT AAA CAG CCT	114
Met Lys Glu Ser Lys Gln Pro	
1 5	
GCC TCA CAA CTC CAA AAA CAA AAG GGA GAT ACT CCA GCT TCA GCA ACA	162
Ala Ser Gln Leu Gln Lys Gln Lys Gly Asp Thr Pro Ala Ser Ala Thr	
10 15 20	
GCA CCT ACA GAA GCG GCT CAA ATT ATT TCT GCA GCA GGT GAT ACC CTG	210
Ala Pro Thr Glu Ala Ala Gln Ile Ile Ser Ala Ala Gly Asp Thr Leu	
25 30 35	
TCG GTC CCA GCC CCT GCA GTT CAG CCT GAG GAA TCT TTA AAA ACT GAT	258
Ser Val Pro Ala Pro Ala Val Gln Pro Glu Glu Ser Leu Lys Thr Asp	
40 45 50 55	
CAC CCT GAA ATT GGT GAA GGA AAA CCC ACA CCT GCA CTT TCA GAA GCA	306
His Pro Glu Ile Gly Glu Gly Lys Pro Thr Pro Ala Leu Ser Glu Ala	
60 65 70	
TCC TCA TCT TCT ATA AGG GAG CGA CCA CCT GAA GAA GTT GCA GCT CGC	354
Ser Ser Ser Ser Ile Arg Glu Arg Pro Pro Glu Glu Val Ala Ala Arg	
75 80 85	
CTT GCA CAA CAG GAA AAA CAA GAA CAA GTT AAA ATT GAG TCT CTA GCC	402
Leu Ala Gln Gln Glu Lys Gln Glu Gln Val Lys Ile Glu Ser Leu Ala	
90 95 100	
AAG AGC TTA GAA GAT GCT CTG AGG CAA ACT GCA AGT GTC ACT CTG CAG	450
Lys Ser Leu Glu Asp Ala Leu Arg Gln Thr Ala Ser Val Thr Leu Gln	
105 110 115	
GCT ATT GCA GCT CAG AAT GCT GCG GTC CAG GCT GTC AAT GCA CAC TCC	498
Ala Ile Ala Ala Gln Asn Ala Ala Val Gln Ala Val Asn Ala His Ser	
120 125 130 135	

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AAC ATA TTG AAA GCC GCC ATG GAC AAT TCT GAG ATT GCA GGC GAG AAG Asn Ile Leu Lys Ala Ala Met Asp Asn Ser Glu Ile Ala Gly Glu Lys 140 145 150	546
AAA TCT GCT CAG TGG CGC ACA GTG GAG GGT GCA TTG AAG GAA CGC AGA Lys Ser Ala Gln Trp Arg Thr Val Glu Gly Ala Leu Lys Glu Arg Arg 155 160 165	594
AAG GCA GTA GAT GAA GCT GCC GAT GCC CTT CTC AAA GCC AAA GAA GAG Lys Ala Val Asp Glu Ala Ala Asp Ala Leu Leu Lys Ala Lys Glu Glu 170 175 180	642
TTA GAG AAG ATG AAA AGT GTG ATT GAA AAT GCA AAG AAA AAA GAG GTT Leu Glu Lys Met Lys Ser Val Ile Glu Asn Ala Lys Lys Lys Glu Val 185 190 195	690
GCT GGG GCC AAG CCT CAT ATA ACT GCT GCA GAG GGT AAA CTT CAC AAC Ala Gly Ala Lys Pro His Ile Thr Ala Ala Glu Gly Lys Leu His Asn 200 205 210 215	738
ATG ATA GTT GAT CTG GAT AAT GTG GTC AAA AAG GTC CAA GCA GCT CAG Met Ile Val Asp Leu Asp Asn Val Val Lys Lys Val Gln Ala Ala Gln 220 225 230	786
TCT GAG GCT AAG GTT GTA TCT CAG TAT CAT GAG CTG GTG GTC CAA GCT Ser Glu Ala Lys Val Val Ser Gln Tyr His Glu Leu Val Val Gln Ala 235 240 245	834
CGG GAT GAC TTT AAA CGA GAG CTG GAC AGT ATT ACT CCA GAA GTC CTT Arg Asp Asp Phe Lys Arg Glu Leu Asp Ser Ile Thr Pro Glu Val Leu 250 255 260	882
CCT GGG TGG AAA GGA ATG AGT GTT TCA GAC TTA GCT GAC AAG CTC TCT Pro Gly Trp Lys Gly Met Ser Val Ser Asp Leu Ala Asp Lys Leu Ser 265 270 275	930
ACT GAT GAT CTG AAC TCC CTC ATT GCT CAT GCA CAT CGT CGT ATT GAT Thr Asp Asp Leu Asn Ser Leu Ile Ala His Ala His Arg Arg Ile Asp 280 285 290 295	978
CAG CTG AAC AGA GAG CTG GCA GAA CAG AAG GCC ACC GAA AAG CAG CAC Gln Leu Asn Arg Glu Leu Ala Glu Gln Lys Ala Thr Glu Lys Gln His 300 305 310	1026
ATC ACG TTA GCC TTG GAG AAA CAA AAG CTG GAA GAA AAG CGG GCA TTT Ile Thr Leu Ala Leu Glu Lys Gln Lys Leu Glu Glu Lys Arg Ala Phe 315 320 325	1074
GAC TCT GCA GTA GCA AAA GCA TTA GAA CAT CAC AGA AGT GAA ATA CAG Asp Ser Ala Val Ala Lys Ala Leu Glu His His Arg Ser Glu Ile Gln 330 335 340	1122

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GCT GAA CAG GAC AGA AAG ATA GAA GAA GTC AGA GAT GCC ATG GAA AAT Ala Glu Gln Asp Arg Lys Ile Glu Glu Val Arg Asp Ala Met Glu Asn 345 350 355	1170
GAA ATG AGA ACC CCT TCG CCG ACA GCA GCT GCC CAC ACT GAT CAC TTG Glu Met Arg Thr Pro Ser Pro Thr Ala Ala Ala His Thr Asp His Leu 360 365 370 375	1218
CGA GAT GTC CTT AGG GTA CAA GAA CAG GAA TTG AAG TCT GAA TTT GAG Arg Asp Val Leu Arg Val Gln Glu Gln Glu Leu Lys Ser Glu Phe Glu 380 385 390	1266
CAG AAC CTG TCT GAG AAA CTC TCT GAA CAA GAA TTA CAA TTT CGT CGT Gln Asn Leu Ser Glu Lys Leu Ser Glu Gln Glu Leu Gln Phe Arg Arg 395 400 405	1314
CTC AGT CAA GAG CAA GTT GAC AAC TTT ACT CTG GAT ATA AAT ACT GCC Leu Ser Gln Glu Gln Val Asp Asn Phe Thr Leu Asp Ile Asn Thr Ala 410 415 420	1362
TAT GCC AGA CTC AGA GGA ATC GAA CAG GCT GTT CAG AGC CAT GCA GTT Tyr Ala Arg Leu Arg Gly Ile Glu Gln Ala Val Gln Ser His Ala Val 425 430 435	1410
GCT GAA GAG GAA GCC AGA AAA GCC CAC CAA CTC TGG CTT TCA GTG GAG Ala Glu Glu Glu Ala Arg Lys Ala His Gln Leu Trp Leu Ser Val Glu 440 445 450 455	1458
GCA TTA AAG TAC AGC ATG AAG ACC TCA TCT GCA GAA ACA CCT ACT ATC Ala Leu Lys Tyr Ser Met Lys Thr Ser Ser Ala Glu Thr Pro Thr Ile 460 465 470	1506
CCG CTG GGT AGT GCG GTT GAG GCC ATC AAA GCC AAC TGT TCT GAT AAT Pro Leu Gly Ser Ala Val Glu Ala Ile Lys Ala Asn Cys Ser Asp Asn 475 480 485	1554
GAA TTC ACC CAA GCT TTA ACC GCA GCT ATC CCT CCA GAG TCC CTG ACC Glu Phe Thr Gln Ala Leu Thr Ala Ala Ile Pro Pro Glu Ser Leu Thr 490 495 500	1602
CGT GGG GTG TAC AGT GAA GAG ACC CTT AGA GCC CGT TTC TAT GCT GTT Arg Gly Val Tyr Ser Glu Glu Thr Leu Arg Ala Arg Phe Tyr Ala Val 505 510 515	1650
CAA AAA CTG GCC CGA AGG GTA GCA ATG ATT GAT GAA ACC AGA AAT AGC Gln Lys Leu Ala Arg Arg Val Ala Met Ile Asp Glu Thr Arg Asn Ser 520 525 530 535	1698
TTG TAC CAG TAC TTC CTC TCC TAC CTA CAG TCC CTG CTC CTA TTC CCA Leu Tyr Gln Tyr Phe Leu Ser Tyr Leu Gln Ser Leu Leu Leu Phe Pro 540 545 550	1746

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CCT CAG CAA CTG AAG CCG CCC CCA GAG CTC TGC CCT GAG GAT ATA AAC Pro Gln Gln Leu Lys Pro Pro Pro Glu Leu Cys Pro Glu Asp Ile Asn 555 560 565	1794
ACA TTT AAA TTA CTG TCA TAT GCT TCC TAT TGC ATT GAG CAT GGT GAT Thr Phe Lys Leu Leu Ser Tyr Ala Ser Tyr Cys Ile Glu His Gly Asp 570 575 580	1842
CTG GAG CTA GCA GCA AAG TTT GTC AAT CAG CTG AAG GGG GAA TCC AGA Leu Glu Leu Ala Ala Lys Phe Val Asn Gln Leu Lys Gly Glu Ser Arg 585 590 595	1890
CGA GTG GCA CAG GAC TGG CTG AAG GAA GCC CGA ATG ACC CTA GAA ACG Arg Val Ala Gln Asp Trp Leu Lys Glu Ala Arg Met Thr Leu Glu Thr 600 605 610 615	1938
AAA CAG ATA GTG GAA ATC CTG ACA GCA TAT GCC AGC GCC GTA GGA ATA Lys Gln Ile Val Glu Ile Leu Thr Ala Tyr Ala Ser Ala Val Gly Ile 620 625 630	1986
GGA ACC ACT CAG GTG CAG CCA GAG TGAGGTTT AG GAAGATTTTC ATAAAGTCAT Gly Thr Thr Gln Val Gln Pro Glu 635	2040
ATTTCATGTC AAAGGAAATC AGCAGTGATA GATGAAGGGT TCGCAGCGAG AGTCCCGGAC	2100
TTGTCTAGAA ATGAGCAGGT TTACAAGTAC TGTTCTAAAT GTTAACACCT GTTGCAATTTA	2160
TATTCTTTCC ATTGCTATC ATGTCAGTGA ACGCCAGGAG TGCTTTCTTT GCAACTTGTC	2220
TAACATTTTC TGTTTTTTCA GGTTTTACTG ATGAGGCTTG TGAGGCCAAT CAAAATAATG	2280
TTTGTGATCT CTACTACTGT TGATTTTGCC CTCGAGCAA ACTGAATAAA GCAACAAGAT	2340
GAAAAAAAAA AAAAAAAAAA	2360

(2) INFORMATION FOR SEQ ID NO:2:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 639 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met	Lys	Glu	Ser	Lys	Gln	Pro	Ala	Ser	Gln	Leu	Gln	Lys	Gln	Lys	Gly
1				5					10					15	
Asp	Thr	Pro	Ala	Ser	Ala	Thr	Ala	Pro	Thr	Glu	Ala	Ala	Gln	Ile	Ile
			20					25					30		

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Ser	Ala	Ala	Gly	Asp	Thr	Leu	Ser	Val	Pro	Ala	Pro	Ala	Val	Gln	Pro
	35						40					45			
Glu	Glu	Ser	Leu	Lys	Thr	Asp	His	Pro	Glu	Ile	Gly	Glu	Gly	Lys	Pro
	50					55					60				
Thr	Pro	Ala	Leu	Ser	Glu	Ala	Ser	Ser	Ser	Ser	Ile	Arg	Glu	Arg	Pro
	65				70					75					80
Pro	Glu	Glu	Val	Ala	Ala	Arg	Leu	Ala	Gln	Gln	Glu	Lys	Gln	Glu	Gln
				85					90					95	
Val	Lys	Ile	Glu	Ser	Leu	Ala	Lys	Ser	Leu	Glu	Asp	Ala	Leu	Arg	Gln
			100				105						110		
Thr	Ala	Ser	Val	Thr	Leu	Gln	Ala	Ile	Ala	Ala	Gln	Asn	Ala	Ala	Val
		115					120					125			
Gln	Ala	Val	Asn	Ala	His	Ser	Asn	Ile	Leu	Lys	Ala	Ala	Met	Asp	Asn
	130					135					140				
Ser	Glu	Ile	Ala	Gly	Glu	Lys	Lys	Ser	Ala	Gln	Trp	Arg	Thr	Val	Glu
	145				150					155					160
Gly	Ala	Leu	Lys	Glu	Arg	Arg	Lys	Ala	Val	Asp	Glu	Ala	Ala	Asp	Ala
				165					170					175	
Leu	Leu	Lys	Ala	Lys	Glu	Glu	Leu	Glu	Lys	Met	Lys	Ser	Val	Ile	Glu
			180					185					190		
Asn	Ala	Lys	Lys	Lys	Glu	Val	Ala	Gly	Ala	Lys	Pro	His	Ile	Thr	Ala
		195					200					205			
Ala	Glu	Gly	Lys	Leu	His	Asn	Met	Ile	Val	Asp	Leu	Asp	Asn	Val	Val
	210					215					220				
Lys	Lys	Val	Gln	Ala	Ala	Gln	Ser	Glu	Ala	Lys	Val	Val	Ser	Gln	Tyr
	225				230					235					240
His	Glu	Leu	Val	Val	Gln	Ala	Arg	Asp	Asp	Phe	Lys	Arg	Glu	Leu	Asp
				245					250					255	
Ser	Ile	Thr	Pro	Glu	Val	Leu	Pro	Gly	Trp	Lys	Gly	Met	Ser	Val	Ser
			260					265					270		
Asp	Leu	Ala	Asp	Lys	Leu	Ser	Thr	Asp	Asp	Leu	Asn	Ser	Leu	Ile	Ala
		275					280					285			
His	Ala	His	Arg	Arg	Ile	Asp	Gln	Leu	Asn	Arg	Glu	Leu	Ala	Glu	Gln
	290					295					300				

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Lys Ala Thr Glu Lys Gln His Ile Thr Leu Ala Leu Glu Lys Gln Lys
 305 310 315 320
 Leu Glu Glu Lys Arg Ala Phe Asp Ser Ala Val Ala Lys Ala Leu Glu
 325 330 335
 His His Arg Ser Glu Ile Gln Ala Glu Gln Asp Arg Lys Ile Glu Glu
 340 345 350
 Val Arg Asp Ala Met Glu Asn Glu Met Arg Thr Pro Ser Pro Thr Ala
 355 360 365
 Ala Ala His Thr Asp His Leu Arg Asp Val Leu Arg Val Gln Glu Gln
 370 375 380
 Glu Leu Lys Ser Glu Phe Glu Gln Asn Leu Ser Glu Lys Leu Ser Glu
 385 390 395 400
 Gln Glu Leu Gln Phe Arg Arg Leu Ser Gln Glu Gln Val Asp Asn Phe
 405 410 415
 Thr Leu Asp Ile Asn Thr Ala Tyr Ala Arg Leu Arg Gly Ile Glu Gln
 420 425 430
 Ala Val Gln Ser His Ala Val Ala Glu Glu Glu Ala Arg Lys Ala His
 435 440 445
 Gln Leu Trp Leu Ser Val Glu Ala Leu Lys Tyr Ser Met Lys Thr Ser
 450 455 460
 Ser Ala Glu Thr Pro Thr Ile Pro Leu Gly Ser Ala Val Glu Ala Ile
 465 470 475 480
 Lys Ala Asn Cys Ser Asp Asn Glu Phe Thr Gln Ala Leu Thr Ala Ala
 485 490 495
 Ile Pro Pro Glu Ser Leu Thr Arg Gly Val Tyr Ser Glu Glu Thr Leu
 500 505 510
 Arg Ala Arg Phe Tyr Ala Val Gln Lys Leu Ala Arg Arg Val Ala Met
 515 520 525
 Ile Asp Glu Thr Arg Asn Ser Leu Tyr Gln Tyr Phe Leu Ser Tyr Leu
 530 535 540
 Gln Ser Leu Leu Leu Phe Pro Pro Gln Gln Leu Lys Pro Pro Pro Glu
 545 550 555 560
 Leu Cys Pro Glu Asp Ile Asn Thr Phe Lys Leu Leu Ser Tyr Ala Ser
 565 570 575

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ATG	ACA	CTC	CAC	GCC	ACC	CGG	GGG	GCT	GCA	CTC	CTC	TCT	TGG	GTG	AAC	48
Met	Thr	Leu	His	Ala	Thr	Arg	Gly	Ala	Ala	Leu	Leu	Ser	Trp	Val	Asn	
1				5					10					15		
AGT	CTA	CAC	GTG	GCT	GAC	CCT	GTG	GAG	GCT	GTG	CTG	CAG	CTC	CAG	GAC	96
Ser	Leu	His	Val	Ala	Asp	Pro	Val	Glu	Ala	Val	Leu	Gln	Leu	Gln	Asp	
			20					25					30			
TGC	AGC	ATC	TTC	ATC	AAG	ATC	ATT	GAC	AGA	ATC	CAT	GGC	ACT	GAA	GAG	144
Cys	Ser	Ile	Phe	Ile	Lys	Ile	Ile	Asp	Arg	Ile	His	Gly	Thr	Glu	Glu	
		35					40					45				

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GGA CAG CAA ATC TTG AAG CAG CCG GTG TCA GAG AGA CTG GAC TTT GTG Gly Gln Gln Ile Leu Lys Gln Pro Val Ser Glu Arg Leu Asp Phe Val 50 55 60	192
TGC AGT TTT CTG CAG AAA AAT CGA AAA CAT CCC TCT TCC CCA GAA TGC Cys Ser Phe Leu Gln Lys Asn Arg Lys His Pro Ser Ser Pro Glu Cys 65 70 75 80	240
CTG GTA TCT GCA CAG AAG GTG CTA GAG GGA TCA GAG CTG GAA CTG GCG Leu Val Ser Ala Gln Lys Val Leu Glu Gly Ser Glu Leu Glu Leu Ala 85 90 95	288
AAG ATG ACC ATG CTG CTC TTA TAC CAC TCT ACC ATG AGC TCC AAA AGT Lys Met Thr Met Leu Leu Leu Tyr His Ser Thr Met Ser Ser Lys Ser 100 105 110	336
CCC AGG GAC TGG GAA CAG TTT GAA TAT AAA ATT CAG GCT GAG TTG GCT Pro Arg Asp Trp Glu Gln Phe Glu Tyr Lys Ile Gln Ala Glu Leu Ala 115 120 125	384
GTC ATT CTT AAA TTT GTG CTG GAC CAT GAG GAC GGG CTA AAC CTT AAT Val Ile Leu Lys Phe Val Leu Asp His Glu Asp Gly Leu Asn Leu Asn 130 135 140	432
GAG GAC CTA GAG AAC TTC CTA CAG AAA GCT CCT GTG CCT TCT ACC TGT Glu Asp Leu Glu Asn Phe Leu Gln Lys Ala Pro Val Pro Ser Thr Cys 145 150 155 160	480
TCT AGC ACA TTC CCT GAA GAG CTC TCC CCA CCT AGC CAC CAG GCC AAG Ser Ser Thr Phe Pro Glu Glu Leu Ser Pro Pro Ser His Gln Ala Lys 165 170 175	528
AGG GAG ATT CGC TTC CTA GAG CTA CAG AAG GTT GCC TCC TCT TCC AGT Arg Glu Ile Arg Phe Leu Glu Leu Gln Lys Val Ala Ser Ser Ser Ser 180 185 190	576
GGG AAC AAC TTT CTC TCA GGT TCT CCA GCT TCT CCC ATG GGT GAT ATC Gly Asn Asn Phe Leu Ser Gly Ser Pro Ala Ser Pro Met Gly Asp Ile 195 200 205	624
CTG CAG ACC CCA CAG TTC CAG ATG AGA CGG CTG AAG AAG CAG CTT GCT Leu Gln Thr Pro Gln Phe Gln Met Arg Arg Leu Lys Lys Gln Leu Ala 210 215 220	672
GAT GAG AGA AGT AAT AGG GAT GAG CTG GAG CTG GAG CTA GCT GAG AAC Asp Glu Arg Ser Asn Arg Asp Glu Leu Glu Leu Glu Leu Ala Glu Asn 225 230 235 240	720
CGC AAG CTC CTC ACC GAG AAG GAT GCA CAG ATA GCC ATG ATG CAG CAG Arg Lys Leu Leu Thr Glu Lys Asp Ala Gln Ile Ala Met Met Gln Gln 245 250 255	768

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CGC ATT GAC CGC CTA GCC CTG CTG AAT GAG AAG CAG GCG GCC AGC CCA Arg Ile Asp Arg Leu Ala Leu Leu Asn Glu Lys Gln Ala Ala Ser Pro 260 265 270	816
CTG GAG CCC AAG GAG CTT GAG GAG CTG CGT GAC AAG AAT GAG AGC CTT Leu Glu Pro Lys Glu Leu Glu Glu Leu Arg Asp Lys Asn Glu Ser Leu 275 280 285	864
ACC ATG CGG CTG CAT GAA ACC CTG AAG CAG TGC CAG GAC CTG AAG ACA Thr Met Arg Leu His Glu Thr Leu Lys Gln Cys Gln Asp Leu Lys Thr 290 295 300	912
GAG AAG AGC CAG ATG GAT CGC AAA ATC AAC CAG CTT TCG GAG GAG AAT Glu Lys Ser Gln Met Asp Arg Lys Ile Asn Gln Leu Ser Glu Glu Asn 305 310 315 320	960
GGA GAC CTT TCC TTT AAG CTG CGG GAG TTT GCC AGT CAT CTG CAG CAG Gly Asp Leu Ser Phe Lys Leu Arg Glu Phe Ala Ser His Leu Gln Gln 325 330 335	1008
CTA CAG GAT GCC CTC AAT GAG CTG ACG GAG GAG CAC AGC AAG GCC ACT Leu Gln Asp Ala Leu Asn Glu Leu Thr Glu Glu His Ser Lys Ala Thr 340 345 350	1056
CAG GAG TGG CTA GAG AAG CAG GCC CAG CTG GAG AAG GAG CTC AGC GCA Gln Glu Trp Leu Glu Lys Gln Ala Gln Leu Glu Lys Glu Leu Ser Ala 355 360 365	1104
GCC CTG CAG GAC AAG AAA TGC CTT GAA GAG AAG AAC GAA ATC CTT CAG Ala Leu Gln Asp Lys Lys Cys Leu Glu Glu Lys Asn Glu Ile Leu Gln 370 375 380	1152
GGA AAA CTT TCA CAG CTG GAA GAA CAC TTG TCC CAG CTG CAG GAT AAC Gly Lys Leu Ser Gln Leu Glu Glu His Leu Ser Gln Leu Gln Asp Asn 385 390 395 400	1200
CCA CCC CAG GAG AAG GGC GAG GTG CTG GGT GAT GTC TTG CAG CTG GAA Pro Pro Gln Glu Lys Gly Glu Val Leu Gly Asp Val Leu Gln Leu Glu 405 410 415	1248
ACC TTG AAG CAA GAG GCA GCC ACT CTT GCT GCA AAC AAC ACA CAG CTC Thr Leu Lys Gln Glu Ala Ala Thr Leu Ala Ala Asn Asn Thr Gln Leu 420 425 430	1296
CAA GCC AGG GTA GAG ATG CTG GAG ACT GAG CGA GGC CAG CAG GAA GCC Gln Ala Arg Val Glu Met Leu Glu Thr Glu Arg Gly Gln Gln Glu Ala 435 440 445	1344
AAG CTG CTT GCT GAG CGG GGC CAC TTC GAA GAA GAA AAG CAG CAG CTG Lys Leu Leu Ala Glu Arg Gly His Phe Glu Glu Glu Lys Gln Gln Leu 450 455 460	1392

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TCT AGC CTG ATC ACT GAC CTG CAG AGC TCC ATC TCC AAC CTC AGC CAG Ser Ser Leu Ile Thr Asp Leu Gln Ser Ser Ile Ser Asn Leu Ser Gln 465 470 475 480	1440
GCC AAG GAA GAG CTG GAG CAG GCC TCC CAG GCT CAT GGG GCC CGG TTG Ala Lys Glu Glu Leu Glu Gln Ala Ser Gln Ala His Gly Ala Arg Leu 485 490 495	1488
ACT GCC CAG GTG GCC TCT CTG ACC TCT GAG CTC ACC ACA CTC AAT GCC Thr Ala Gln Val Ala Ser Leu Thr Ser Glu Leu Thr Thr Leu Asn Ala 500 505 510	1536
ACC ATC CAG CAA CAG GAT CAA GAA CTG GCT GGC CTG AAG CAG CAG GCC Thr Ile Gln Gln Gln Asp Gln Glu Leu Ala Gly Leu Lys Gln Gln Ala 515 520 525	1584
AAA GAG AAG CAG GCC CAG CTA GCA CAG ACC CTC CAA CAG CAA GAA CAG Lys Glu Lys Gln Ala Gln Leu Ala Gln Thr Leu Gln Gln Gln Glu Gln 530 535 540	1632
GCC TCC CAG GGC CTC CGC CAC CAG GTG GAG CAG CTA AGC AGT AGC CTG Ala Ser Gln Gly Leu Arg His Gln Val Glu Gln Leu Ser Ser Ser Leu 545 550 555 560	1680
AAG CAG AAG GAG CAG CAG TTG AAG GAG GTA GCG GAG AAG CAG GAG GCA Lys Gln Lys Glu Gln Gln Leu Lys Glu Val Ala Glu Lys Gln Glu Ala 565 570 575	1728
ACT AGG CAG GAC CAT GCC CAG CAA CTG GCC ACT GCT GCA GAG GAG CGA Thr Arg Gln Asp His Ala Gln Gln Leu Ala Thr Ala Ala Glu Glu Arg 580 585 590	1776
GAG GCC TCC TTA AGG GAG CGG GAT GCG GCT CTC AAG CAG CTG GAG GCA Glu Ala Ser Leu Arg Glu Arg Asp Ala Ala Leu Lys Gln Leu Glu Ala 595 600 605	1824
CTG GAG AAG GAG AAG GCT GCC AAG CTG GAG ATT CTG CAG CAG CAA CTT Leu Glu Lys Glu Lys Ala Ala Lys Leu Glu Ile Leu Gln Gln Gln Leu 610 615 620	1872
CAG GTG GCT AAT GAA GCC CGG GAC AGT GCC CAG ACC TCA GTG ACA CAG Gln Val Ala Asn Glu Ala Arg Asp Ser Ala Gln Thr Ser Val Thr Gln 625 630 635 640	1920
GCC CAG CGG GAG AAG GCA GAG CTG AGC CGG AAG GTG GAG GAA CTC CAG Ala Gln Arg Glu Lys Ala Glu Leu Ser Arg Lys Val Glu Glu Leu Gln 645 650 655	1968
GCC TGT GTT GAG ACA GCC CGC CAG GAA CAG CAT GAG GCC CAG GCC CAG Ala Cys Val Glu Thr Ala Arg Gln Glu Gln His Glu Ala Gln Ala Gln 660 665 670	2016

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GTT GCA GAG CTA GAG TTG CAG CTG CGG TCT GAG CAG CAA AAA GCA ACT Val Ala Glu Leu Glu Leu Gln Leu Arg Ser Glu Gln Gln Lys Ala Thr 675 680 685	2064
GAG AAA GAA AGG GTG GCC CAG GAG AAG GAC CAG CTC CAG GAG CAG CTC Glu Lys Glu Arg Val Ala Gln Glu Lys Asp Gln Leu Gln Glu Gln Leu 690 695 700	2112
CAG GCC CTC AAA GAG TCC TTG AAG GTC ACC AAG GGC AGC CTT GAA GAG Gln Ala Leu Lys Glu Ser Leu Lys Val Thr Lys Gly Ser Leu Glu Glu 705 710 715 720	2160
GAG AAG CGC AGG GCT GCA GAT GCC CTG GAA GAG CAG CAG CGT TGT ATC Glu Lys Arg Arg Ala Ala Asp Ala Leu Glu Glu Gln Gln Arg Cys Ile 725 730 735	2208
TCT GAG CTG AAG GCA GAG ACC CGA AGC CTG GTG GAG CAG CAT AAG CGG Ser Glu Leu Lys Ala Glu Thr Arg Ser Leu Val Glu Gln His Lys Arg 740 745 750	2256
GAA CGA AAG GAG CTG GAA GAA GAG AGG GCT GGG CGC AAG GGG CTG GAG Glu Arg Lys Glu Leu Glu Glu Glu Arg Ala Gly Arg Lys Gly Leu Glu 755 760 765	2304
GCT CGA TTA CTG CAG CTT GGG GAG GCC CAT CAG GCT GAG ACT GAA GTC Ala Arg Leu Leu Gln Leu Gly Glu Ala His Gln Ala Glu Thr Glu Val 770 775 780	2352
CTG CGG CGG GAG CTG GCA GAG GCC ATG GCT GCC CAG CAC ACA GCT GAG Leu Arg Arg Glu Leu Ala Glu Ala Met Ala Ala Gln His Thr Ala Glu 785 790 795 800	2400
AGT GAG TGT GAG CAG CTC GTC AAA GAA GTA GCT GCC TGG CGT GAC GGG Ser Glu Cys Glu Gln Leu Val Lys Glu Val Ala Ala Trp Arg Asp Gly 805 810 815	2448
TAT GAG GAT AGC CAG CAA GAG GAG GCA CAG TAT GGC GCC ATG TTC CAG Tyr Glu Asp Ser Gln Gln Glu Glu Ala Gln Tyr Gly Ala Met Phe Gln 820 825 830	2496
GAA CAG CTG ATG ACT TTG AAG GAG GAA TGT GAG AAG GCC CGC CAG GAG Glu Gln Leu Met Thr Leu Lys Glu Glu Cys Glu Lys Ala Arg Gln Glu 835 840 845	2544
CTG CAG GAG GCA AAG GAG AAG GTG GCA GGC ATA GAA TCC CAC AGC GAG Leu Gln Glu Ala Lys Glu Lys Val Ala Gly Ile Glu Ser His Ser Glu 850 855 860	2592
CTC CAG ATA AGC CGG CAG CAG AAC AAA CTA GCT GAG CTC CAT GCC AAC Leu Gln Ile Ser Arg Gln Gln Asn Lys Leu Ala Glu Leu His Ala Asn 865 870 875 880	2640

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CTG GCC AGA GCA CTC CAG CAG GTC CAA GAG AAG GAA GTC AGG GCC CAG Leu Ala Arg Ala Leu Gln Gln Val Gln Glu Lys Glu Val Arg Ala Gln 885 890 895	2688
AAG CTT GCA GAT GAC CTC TCC ACT CTG CAG GAA AAG ATG GCT GCC ACC Lys Leu Ala Asp Asp Leu Ser Thr Leu Gln Glu Lys Met Ala Ala Thr 900 905 910	2736
AGC AAA GAG GTG GCC CGC TTG GAG ACC TTG GTC CGC AAG GCA GGT GAG Ser Lys Glu Val Ala Arg Leu Glu Thr Leu Val Arg Lys Ala Gly Glu 915 920 925	2784
CAG CAG GAA ACA GCC TCC CGG GAG TTA GTC AAG GAG CCT GCG AGG GCA Gln Gln Glu Thr Ala Ser Arg Glu Leu Val Lys Glu Pro Ala Arg Ala 930 935 940	2832
GGA GAC AGA CAG CCC GAG TGG CTG GAA GAG CAA CAG GGA CGC CAG TTC Gly Asp Arg Gln Pro Glu Trp Leu Glu Glu Gln Gln Gly Arg Gln Phe 945 950 955 960	2880
TGC AGC ACA CAG GCA GCG CTG CAG GCT ATG GAG CGG GAG GCA GAG CAG Cys Ser Thr Gln Ala Ala Leu Gln Ala Met Glu Arg Glu Ala Glu Gln 965 970 975	2928
ATG GGC AAT GAG CTG GAA CGG CTG CGG GCC GCG CTG ATG GAG AGC CAG Met Gly Asn Glu Leu Glu Arg Leu Arg Ala Ala Leu Met Glu Ser Gln 980 985 990	2976
GGG CAG CAG CAG GAG GAG CGT GGG CAG CAG GAA AGG GAG GTG GCG CGG Gly Gln Gln Gln Glu Glu Arg Gly Gln Gln Glu Arg Glu Val Ala Arg 995 1000 1005	3024
CTG ACC CAG GAG CGG GGC CGT GCC CAG GCT GAC CTT GCC CTG GAG AAG Leu Thr Gln Glu Arg Gly Arg Ala Gln Ala Asp Leu Ala Leu Glu Lys 1010 1015 1020	3072
GCG GCC AGA GCA GAG CTT GAG ATG CGG CTG CAG AAC GCC CTC AAC GAG Ala Ala Arg Ala Glu Leu Glu Met Arg Leu Gln Asn Ala Leu Asn Glu 1025 1030 1035 1040	3120
CAG CGT GTG GAG TTC GCT ACC CTG CAA GAG GCA CTG GCT CAT GCC CTG Gln Arg Val Glu Phe Ala Thr Leu Gln Glu Ala Leu Ala His Ala Leu 1045 1050 1055	3168
ACG GAA AAG GAA GGC AAG GAC CAG GAG TTG GCC AAG CTT CGT GGT CTG Thr Glu Lys Glu Gly Lys Asp Gln Glu Leu Ala Lys Leu Arg Gly Leu 1060 1065 1070	3216
GAG GCA GCC CAG ATA AAA GAG CTG GAG GAA CTT CGG CAA ACC GTG AAG Glu Ala Ala Gln Ile Lys Glu Leu Glu Glu Leu Arg Gln Thr Val Lys 1075 1080 1085	3264

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CAA CTG AAG GAA CAG CTG GCT AAG AAA GAA AAG GAG CAC GCA TCT GGC Gln Leu Lys Glu Gln Leu Ala Lys Lys Glu Lys Glu His Ala Ser Gly 1090 1095 1100	3312
TCA GGA GCC CAA TCT GAG GCT GCT GGC AGG ACA GAG CCA ACA GGC CCC Ser Gly Ala Gln Ser Glu Ala Ala Gly Arg Thr Glu Pro Thr Gly Pro 1105 1110 1115 1120	3360
AAG CTG GAA GCA CTG CGG GCA GAG GTG AGC AAG CTG GAA CAG CAA TGC Lys Leu Glu Ala Leu Arg Ala Glu Val Ser Lys Leu Glu Gln Gln Cys 1125 1130 1135	3408
CAG AAG CAG CAG GAG CAG GCT GAC AGC CTG GAA CGC AGC CTC GAG GCT Gln Lys Gln Gln Glu Gln Ala Asp Ser Leu Glu Arg Ser Leu Glu Ala 1140 1145 1150	3456
GAG CGG GCC TCC CGG GCT GAG CGG GAC AGT GCT CTG GAG ACT CTG CAG Glu Arg Ala Ser Arg Ala Glu Arg Asp Ser Ala Leu Glu Thr Leu Gln 1155 1160 1165	3504
GGC CAG TTA GAG GAG AAG GCC CAG GAG CTA GGG CAC AGT CAG AGT GCC Gly Gln Leu Glu Glu Lys Ala Gln Glu Leu Gly His Ser Gln Ser Ala 1170 1175 1180	3552
TTA GCC TCG GCC CAA CGG GAG TTG GCT GCC TTC CGC ACC AAG GTA CAA Leu Ala Ser Ala Gln Arg Glu Leu Ala Ala Phe Arg Thr Lys Val Gln 1185 1190 1195 1200	3600
GAC CAC AGC AAG GCT GAA GAT GAG TGG AAG GCC CAG GTG GCC CGG GGC Asp His Ser Lys Ala Glu Asp Glu Trp Lys Ala Gln Val Ala Arg Gly 1205 1210 1215	3648
CGG CAA GAG GCT GAG AGG AAA AAT AGC CTC ATC AGC AGC TTG GAG GAG Arg Gln Glu Ala Glu Arg Lys Asn Ser Leu Ile Ser Ser Leu Glu Glu 1220 1225 1230	3696
GAG GTG TCC ATC CTG AAT CGC CAG GTC CTG GAG AAG GAG GGG GAG AGC Glu Val Ser Ile Leu Asn Arg Gln Val Leu Glu Lys Glu Gly Glu Ser 1235 1240 1245	3744
AAG GAG TTG AAG CGG CTG GTG ATG GCC GAG TCA GAG AAG AGC CAG AAG Lys Glu Leu Lys Arg Leu Val Met Ala Glu Ser Glu Lys Ser Gln Lys 1250 1255 1260	3792
CTG GAG GAG AGC TGC GCC TGC TGC AGG CAG AGA CAG CCA GCA ACA GTG Leu Glu Glu Ser Cys Ala Cys Cys Arg Gln Arg Gln Pro Ala Thr Val 1265 1270 1275 1280	3840
CCA GAG CTG CAG AAC GCA GCT CTG CTC TGC GGG AGG AGG TGC AGA GCC Pro Glu Leu Gln Asn Ala Ala Leu Leu Cys Gly Arg Arg Cys Arg Ala 1285 1290 1295	3888

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TCC GGG AGG GAG GCT GAG AAA CAG CGG GTG GCT TCA GAG AAC CTG CGG Ser Gly Arg Glu Ala Glu Lys Gln Arg Val Ala Ser Glu Asn Leu Arg 1300 1305 1310	3936
CAG GAG CTG ACC TCA CAG GCT GAG CGT GCG GAG GAG CTG GGC CAA GAA Gln Glu Leu Thr Ser Gln Ala Glu Arg Ala Glu Glu Leu Gly Gln Glu 1315 1320 1325	3984
TTG AAG GCG TGG CAG GAG AAG TTC TTC CAG AAA GAG CAG GCC CTC TCC Leu Lys Ala Trp Gln Glu Lys Phe Phe Gln Lys Glu Gln Ala Leu Ser 1330 1335 1340	4032
ACC CTG CAG CTC GAG CAC ACC AGC ACA CAG GCC CTG GTG AGT GAG CTG Thr Leu Gln Leu Glu His Thr Ser Thr Gln Ala Leu Val Ser Glu Leu 1345 1350 1355 1360	4080
CTG CCA GCT AAG CAC CTC TGC CAG CAG CTG CAG GCC GAG CAG GCC GCT Leu Pro Ala Lys His Leu Cys Gln Gln Leu Gln Ala Glu Gln Ala Ala 1365 1370 1375	4128
GCC GAG AAA CGC CAC CGT GAG GAG CTG GAG CAG AGC AAG CAG GCC GCT Ala Glu Lys Arg His Arg Glu Glu Leu Glu Gln Ser Lys Gln Ala Ala 1380 1385 1390	4176
GGG GGA CTG CGG GCA GAG CTG CTG CGG GCC CAG CGG GAG CTT GGG GAG Gly Gly Leu Arg Ala Glu Leu Leu Arg Ala Gln Arg Glu Leu Gly Glu 1395 1400 1405	4224
CTG ATT CCT CTG CGG CAG AAG GTG GCA GAG CAG GAG CGA ACA GCT CAG Leu Ile Pro Leu Arg Gln Lys Val Ala Glu Gln Glu Arg Thr Ala Gln 1410 1415 1420	4272
CAG CTG CGG GCA GAG AAG GCC AGC TAT GCA GAG CAG CTG AGC ATG CTG Gln Leu Arg Ala Glu Lys Ala Ser Tyr Ala Glu Gln Leu Ser Met Leu 1425 1430 1435 1440	4320
AAG AAG GCG CAT GGC CTG CTG GCA GAG GAG AAC CGG GGG CTG GGT GAG Lys Lys Ala His Gly Leu Leu Ala Glu Glu Asn Arg Gly Leu Gly Glu 1445 1450 1455	4368
CGG GCC AAC CTT GGC CGG CAG TTT CTG GAA GTG GAG TTG GAC CAG GCC Arg Ala Asn Leu Gly Arg Gln Phe Leu Glu Val Glu Leu Asp Gln Ala 1460 1465 1470	4416
CGG GAA AAG TAT GTC CAA GAG TTG GCA GCC GTA CGT GCT GAT GCT GAG Arg Glu Lys Tyr Val Gln Glu Leu Ala Ala Val Arg Ala Asp Ala Glu 1475 1480 1485	4464
ACC CGT CTG GCT GAG GTG CAG CGA GAA GCA CAG AGC ACT GCC CGG GAG Thr Arg Leu Ala Glu Val Gln Arg Glu Ala Gln Ser Thr Ala Arg Glu 1490 1495 1500	4512

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CTG GAG GTG ATG ACT GCC AAG TAT GAG GGT GCC AAG GTC AAG GTC CTG Leu Glu Val Met Thr Ala Lys Tyr Glu Gly Ala Lys Val Lys Val Leu 1505 1510 1515 1520	4560
GAG GAG AGG CAG CGG TTC CAG GAA GAG AGG CAG AAA CTC ACT GCC CAG Glu Glu Arg Gln Arg Phe Gln Glu Glu Arg Gln Lys Leu Thr Ala Gln 1525 1530 1535	4608
GTG GAA GAA CTG AGT AAG AAA CTG GCT GAC TCT GAC CAA GCC AGC AAG Val Glu Glu Leu Ser Lys Lys Leu Ala Asp Ser Asp Gln Ala Ser Lys 1540 1545 1550	4656
GTG CAG CAG CAG AAG CTG AAG GCT GTC CAG GCT CAG GGA GGC GAG AGC Val Gln Gln Gln Lys Leu Lys Ala Val Gln Ala Gln Gly Gly Glu Ser 1555 1560 1565	4704
CAG CAG GAG GCC CAG CGC TTC CAG GCC CAG CTG AAT GAA CTG CAA GCC Gln Gln Glu Ala Gln Arg Phe Gln Ala Gln Leu Asn Glu Leu Gln Ala 1570 1575 1580	4752
CAG TTG AGC CAG AAG GAG CAG GCA GCT GAG CAC TAT AAG CTG CAG ATG Gln Leu Ser Gln Lys Glu Gln Ala Ala Glu His Tyr Lys Leu Gln Met 1585 1590 1595 1600	4800
GAG AAA GCC AAA ACA CAT TAT GAT GCC AAG AAG CAG CAG AAC CAA GAG Glu Lys Ala Lys Thr His Tyr Asp Ala Lys Lys Gln Gln Asn Gln Glu 1605 1610 1615	4848
CTG CAG GAG CAG CTG CGG AGC CTG GAG CAG CTG CAG AAG GAA AAC AAA Leu Gln Glu Gln Leu Arg Ser Leu Glu Gln Leu Gln Lys Glu Asn Lys 1620 1625 1630	4896
GAG CTG CGA GCT GAA GCT GAA CGG CTG GGC CAT GAG CTA CAG CAG GCT Glu Leu Arg Ala Glu Ala Glu Arg Leu Gly His Glu Leu Gln Gln Ala 1635 1640 1645	4944
GGG CTG AAG ACC AAG GAG GCT GAA CAG ACC TGC CGC CAC CTT ACT GCC Gly Leu Lys Thr Lys Glu Ala Glu Gln Thr Cys Arg His Leu Thr Ala 1650 1655 1660	4992
CAG GTG CGC AGC CTG GAG GCA CAG GTT GCC CAT GCA GAC CAG CAG CTT Gln Val Arg Ser Leu Glu Ala Gln Val Ala His Ala Asp Gln Gln Leu 1665 1670 1675 1680	5040
CGA GAC CTG GGC AAA TTC CAG GTG GCA ACT GAT GCT TTA AAG AGC CGT Arg Asp Leu Gly Lys Phe Gln Val Ala Thr Asp Ala Leu Lys Ser Arg 1685 1690 1695	5088
GAG CCC CAG GCT AAG CCC CAG CTG GAC TTG AGT ATT GAC AGC CTG GAT Glu Pro Gln Ala Lys Pro Gln Leu Asp Leu Ser Ile Asp Ser Leu Asp 1700 1705 1710	5136

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CTG AGC TGC GAG GAG GGG ACC CCA CTC AGT ATC ACC AGC AAG CTG CCT Leu Ser Cys Glu Glu Gly Thr Pro Leu Ser Ile Thr Ser Lys Leu Pro 1715 1720 1725	5184
CGT ACC CAG CCA GAC GGC ACC AGC GTC CCT GGA GAA CCA GCC TCA CCT Arg Thr Gln Pro Asp Gly Thr Ser Val Pro Gly Glu Pro Ala Ser Pro 1730 1735 1740	5232
ATC TCC CAG CGC CTG CCC CCC AAG GTA GAA TCC CTG GAG AGT CTC TAC Ile Ser Gln Arg Leu Pro Lys Val Glu Ser Leu Glu Ser Leu Tyr 1745 1750 1755 1760	5280
TTC ACT CCC ATC CCT GCT CGG AGT CAG GCC CCC CTG GAG AGC AGC CTG Phe Thr Pro Ile Pro Ala Arg Ser Gln Ala Pro Leu Glu Ser Ser Leu 1765 1770 1775	5328
GAC TCC CTG GGA GAC GTC TTC CTG GAC TCG GGT CGT AAG ACC CGC TCC Asp Ser Leu Gly Asp Val Phe Leu Asp Ser Gly Arg Lys Thr Arg Ser 1780 1785 1790	5376
GCT CGT CGG CGC ACC ACG CAG ATC ATC AAC ATC ACC ATG ACC AAG AAG Ala Arg Arg Arg Thr Thr Gln Ile Ile Asn Ile Thr Met Thr Lys Lys 1795 1800 1805	5424
CTA GAT GTG GAA GAG CCA GAC AGC GCC AAC TCA TCG TTC TAC AGC ACG Leu Asp Val Glu Glu Pro Asp Ser Ala Asn Ser Ser Phe Tyr Ser Thr 1810 1815 1820	5472
CGG TCT GCT CCT GCT TCC CAG GCT AGC CTG CGA GCC ACC TCC TCT ACT Arg Ser Ala Pro Ala Ser Gln Ala Ser Leu Arg Ala Thr Ser Ser Thr 1825 1830 1835 1840	5520
CAG TCT CTA GCT CGC CTG GGT TCT CCC GAT TAT GGC AAC TCA GCC CTG Gln Ser Leu Ala Arg Leu Gly Ser Pro Asp Tyr Gly Asn Ser Ala Leu 1845 1850 1855	5568
CTC AGC TTG CCT GGC TAC CGC CCC ACC ACT CGC AGT TCT GCT CGT CGT Leu Ser Leu Pro Gly Tyr Arg Pro Thr Thr Arg Ser Ser Ala Arg Arg 1860 1865 1870	5616
TCC CAG GCC GGG GTG TCC AGT GGG GCC CCT CCA GGA AGG AAC AGC TTC Ser Gln Ala Gly Val Ser Ser Gly Ala Pro Pro Gly Arg Asn Ser Phe 1875 1880 1885	5664
TAC ATG GGC ACT TGC CAG GAT GAG CCT GAG CAG CTG GAT GAC TGG AAC Tyr Met Gly Thr Cys Gln Asp Glu Pro Glu Gln Leu Asp Asp Trp Asn 1890 1895 1900	5712
CGC ATT GCA GAG CTG CAG CAG CGC AAT CGA GTG TGC CCC CCA CAT CTG Arg Ile Ala Glu Leu Gln Gln Arg Asn Arg Val Cys Pro Pro His Leu 1905 1910 1915 1920	5760

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AAG ACC TGC TAT CCC CTG GAG TCC AGG CCT TCC CTG AGC CTG GGC ACC Lys Thr Cys Tyr Pro Leu Glu Ser Arg Pro Ser Leu Ser Leu Gly Thr 1925 1930 1935	5808
ATC ACA GAT GAG GAG ATG AAA ACT GGA GAC CCC CAA GAG ACC CTG CGC Ile Thr Asp Glu Glu Met Lys Thr Gly Asp Pro Gln Glu Thr Leu Arg 1940 1945 1950	5856
CGA GCC AGC ATG CAG CCA ATC CAG ATA GCC GAG GGC ACT GGC ATC ACC Arg Ala Ser Met Gln Pro Ile Gln Ile Ala Glu Gly Thr Gly Ile Thr 1955 1960 1965	5904
ACC CGG CAG CAG CGC AAA CGG GTC TCC CTA GAG CCC CAC CAG GGC CCT Thr Arg Gln Gln Arg Lys Arg Val Ser Leu Glu Pro His Gln Gly Pro 1970 1975 1980	5952
GGA ACT CCT GAG TCT AAG AAG GCC ACC AGC TGT TTC CCA CGC CCC ATG Gly Thr Pro Glu Ser Lys Lys Ala Thr Ser Cys Phe Pro Arg Pro Met 1985 1990 1995 2000	6000
ACT CCC CGA GAC CGA CAT GAA GGG CGC AAA CAG AGC ACT ACT GAG GCC Thr Pro Arg Asp Arg His Glu Gly Arg Lys Gln Ser Thr Thr Glu Ala 2005 2010 2015	6048
CAG AAG AAA GCA GCT CCA GCT TCT ACT AAA CAG GCT GAC CGG CGC CAG Gln Lys Lys Ala Ala Pro Ala Ser Thr Lys Gln Ala Asp Arg Arg Gln 2020 2025 2030	6096
TCG ATG GCC TTC AGC ATC CTC AAC ACA CCC AAG AAG CTA GGG AAC AGC Ser Met Ala Phe Ser Ile Leu Asn Thr Pro Lys Lys Leu Gly Asn Ser 2035 2040 2045	6144
CTT CTG CGG CGG GGA GCC TCA AAG AAG GCC CTG TCC AAG GCT TCC CCC Leu Leu Arg Arg Gly Ala Ser Lys Lys Ala Leu Ser Lys Ala Ser Pro 2050 2055 2060	6192
AAC ACT CGC AGT GGA ACC CGC CGT TCT CCG CGC ATT GCC ACC ACC ACA Asn Thr Arg Ser Gly Thr Arg Arg Ser Pro Arg Ile Ala Thr Thr Thr 2065 2070 2075 2080	6240
GCC AGT GCC GCC ACT GCT GCC GCC ATT GGT GCC ACC CCT CGA GCC AAG Ala Ser Ala Ala Thr Ala Ala Ala Ile Gly Ala Thr Pro Arg Ala Lys 2085 2090 2095	6288
GGC AAG GCA AAG CAC TAA Gly Lys Ala Lys His 2100	6306

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(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2101 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```

Met Thr Leu His Ala Thr Arg Gly Ala Ala Leu Leu Ser Trp Val Asn
 1           5           10           15
Ser Leu His Val Ala Asp Pro Val Glu Ala Val Leu Gln Leu Gln Asp
          20           25           30
Cys Ser Ile Phe Ile Lys Ile Ile Asp Arg Ile His Gly Thr Glu Glu
      35           40           45
Gly Gln Gln Ile Leu Lys Gln Pro Val Ser Glu Arg Leu Asp Phe Val
 50           55           60
Cys Ser Phe Leu Gln Lys Asn Arg Lys His Pro Ser Ser Pro Glu Cys
 65           70           75           80
Leu Val Ser Ala Gln Lys Val Leu Glu Gly Ser Glu Leu Glu Leu Ala
          85           90           95
Lys Met Thr Met Leu Leu Leu Tyr His Ser Thr Met Ser Ser Lys Ser
          100          105          110
Pro Arg Asp Trp Glu Gln Phe Glu Tyr Lys Ile Gln Ala Glu Leu Ala
          115          120          125
Val Ile Leu Lys Phe Val Leu Asp His Glu Asp Gly Leu Asn Leu Asn
          130          135          140
Glu Asp Leu Glu Asn Phe Leu Gln Lys Ala Pro Val Pro Ser Thr Cys
          145          150          155          160
Ser Ser Thr Phe Pro Glu Glu Leu Ser Pro Pro Ser His Gln Ala Lys
          165          170          175
Arg Glu Ile Arg Phe Leu Glu Leu Gln Lys Val Ala Ser Ser Ser Ser
          180          185          190
Gly Asn Asn Phe Leu Ser Gly Ser Pro Ala Ser Pro Met Gly Asp Ile
          195          200          205
Leu Gln Thr Pro Gln Phe Gln Met Arg Arg Leu Lys Lys Gln Leu Ala
          210          215          220

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Asp Glu Arg Ser Asn Arg Asp Glu Leu Glu Leu Glu Leu Ala Glu Asn
225 230 235 240

Arg Lys Leu Leu Thr Glu Lys Asp Ala Gln Ile Ala Met Met Gln Gln
245 250 255

Arg Ile Asp Arg Leu Ala Leu Leu Asn Glu Lys Gln Ala Ala Ser Pro
260 265 270

Leu Glu Pro Lys Glu Leu Glu Glu Leu Arg Asp Lys Asn Glu Ser Leu
275 280 285

Thr Met Arg Leu His Glu Thr Leu Lys Gln Cys Gln Asp Leu Lys Thr
290 295 300

Glu Lys Ser Gln Met Asp Arg Lys Ile Asn Gln Leu Ser Glu Glu Asn
305 310 315 320

Gly Asp Leu Ser Phe Lys Leu Arg Glu Phe Ala Ser His Leu Gln Gln
325 330 335

Leu Gln Asp Ala Leu Asn Glu Leu Thr Glu Glu His Ser Lys Ala Thr
340 345 350

Gln Glu Trp Leu Glu Lys Gln Ala Gln Leu Glu Lys Glu Leu Ser Ala
355 360 365

Ala Leu Gln Asp Lys Lys Cys Leu Glu Glu Lys Asn Glu Ile Leu Gln
370 375 380

Gly Lys Leu Ser Gln Leu Glu Glu His Leu Ser Gln Leu Gln Asp Asn
385 390 395 400

Pro Pro Gln Glu Lys Gly Glu Val Leu Gly Asp Val Leu Gln Leu Glu
405 410 415

Thr Leu Lys Gln Glu Ala Ala Thr Leu Ala Ala Asn Asn Thr Gln Leu
420 425 430

Gln Ala Arg Val Glu Met Leu Glu Thr Glu Arg Gly Gln Gln Glu Ala
435 440 445

Lys Leu Leu Ala Glu Arg Gly His Phe Glu Glu Glu Lys Gln Gln Leu
450 455 460

Ser Ser Leu Ile Thr Asp Leu Gln Ser Ser Ile Ser Asn Leu Ser Gln
465 470 475 480

Ala Lys Glu Glu Leu Glu Gln Ala Ser Gln Ala His Gly Ala Arg Leu
485 490 495

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Thr Ala Gln Val Ala Ser Leu Thr Ser Glu Leu Thr Thr Leu Asn Ala
 500 505 510
 Thr Ile Gln Gln Gln Asp Gln Glu Leu Ala Gly Leu Lys Gln Gln Ala
 515 520 525
 Lys Glu Lys Gln Ala Gln Leu Ala Gln Thr Leu Gln Gln Gln Glu Gln
 530 535 540
 Ala Ser Gln Gly Leu Arg His Gln Val Glu Gln Leu Ser Ser Ser Leu
 545 550 555 560
 Lys Gln Lys Glu Gln Gln Leu Lys Glu Val Ala Glu Lys Gln Glu Ala
 565 570 575
 Thr Arg Gln Asp His Ala Gln Gln Leu Ala Thr Ala Ala Glu Glu Arg
 580 585 590
 Glu Ala Ser Leu Arg Glu Arg Asp Ala Ala Leu Lys Gln Leu Glu Ala
 595 600 605
 Leu Glu Lys Glu Lys Ala Ala Lys Leu Glu Ile Leu Gln Gln Gln Leu
 610 615 620
 Gln Val Ala Asn Glu Ala Arg Asp Ser Ala Gln Thr Ser Val Thr Gln
 625 630 635 640
 Ala Gln Arg Glu Lys Ala Glu Leu Ser Arg Lys Val Glu Glu Leu Gln
 645 650 655
 Ala Cys Val Glu Thr Ala Arg Gln Glu Gln His Glu Ala Gln Ala Gln
 660 665 670
 Val Ala Glu Leu Glu Leu Gln Leu Arg Ser Glu Gln Gln Lys Ala Thr
 675 680 685
 Glu Lys Glu Arg Val Ala Gln Glu Lys Asp Gln Leu Gln Glu Gln Leu
 690 695 700
 Gln Ala Leu Lys Glu Ser Leu Lys Val Thr Lys Gly Ser Leu Glu Glu
 705 710 715 720
 Glu Lys Arg Arg Ala Ala Asp Ala Leu Glu Glu Gln Gln Arg Cys Ile
 725 730 735
 Ser Glu Leu Lys Ala Glu Thr Arg Ser Leu Val Glu Gln His Lys Arg
 740 745 750
 Glu Arg Lys Glu Leu Glu Glu Glu Arg Ala Gly Arg Lys Gly Leu Glu
 755 760 765

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Ala Arg Leu Leu Gln Leu Gly Glu Ala His Gln Ala Glu Thr Glu Val
770 775 780

Leu Arg Arg Glu Leu Ala Glu Ala Met Ala Ala Gln His Thr Ala Glu
785 790 795 800

Ser Glu Cys Glu Gln Leu Val Lys Glu Val Ala Ala Trp Arg Asp Gly
805 810 815

Tyr Glu Asp Ser Gln Gln Glu Glu Ala Gln Tyr Gly Ala Met Phe Gln
820 825 830

Glu Gln Leu Met Thr Leu Lys Glu Glu Cys Glu Lys Ala Arg Gln Glu
835 840 845

Leu Gln Glu Ala Lys Glu Lys Val Ala Gly Ile Glu Ser His Ser Glu
850 855 860

Leu Gln Ile Ser Arg Gln Gln Asn Lys Leu Ala Glu Leu His Ala Asn
865 870 875 880

Leu Ala Arg Ala Leu Gln Gln Val Gln Glu Lys Glu Val Arg Ala Gln
885 890 895

Lys Leu Ala Asp Asp Leu Ser Thr Leu Gln Glu Lys Met Ala Ala Thr
900 905 910

Ser Lys Glu Val Ala Arg Leu Glu Thr Leu Val Arg Lys Ala Gly Glu
915 920 925

Gln Gln Glu Thr Ala Ser Arg Glu Leu Val Lys Glu Pro Ala Arg Ala
930 935 940

Gly Asp Arg Gln Pro Glu Trp Leu Glu Glu Gln Gln Gly Arg Gln Phe
945 950 955 960

Cys Ser Thr Gln Ala Ala Leu Gln Ala Met Glu Arg Glu Ala Glu Gln
965 970 975

Met Gly Asn Glu Leu Glu Arg Leu Arg Ala Ala Leu Met Glu Ser Gln
980 985 990

Gly Gln Gln Gln Glu Glu Arg Gly Gln Gln Glu Arg Glu Val Ala Arg
995 1000 1005

Leu Thr Gln Glu Arg Gly Arg Ala Gln Ala Asp Leu Ala Leu Glu Lys
1010 1015 1020

Ala Ala Arg Ala Glu Leu Glu Met Arg Leu Gln Asn Ala Leu Asn Glu
1025 1030 1035 1040

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Gln Arg Val Glu Phe Ala Thr Leu Gln Glu Ala Leu Ala His Ala Leu
1045 1050 1055

Thr Glu Lys Glu Gly Lys Asp Gln Glu Leu Ala Lys Leu Arg Gly Leu
1060 1065 1070

Glu Ala Ala Gln Ile Lys Glu Leu Glu Glu Leu Arg Gln Thr Val Lys
1075 1080 1085

Gln Leu Lys Glu Gln Leu Ala Lys Lys Glu Lys Glu His Ala Ser Gly
1090 1095 1100

Ser Gly Ala Gln Ser Glu Ala Ala Gly Arg Thr Glu Pro Thr Gly Pro
1105 1110 1115 1120

Lys Leu Glu Ala Leu Arg Ala Glu Val Ser Lys Leu Glu Gln Gln Cys
1125 1130 1135

Gln Lys Gln Gln Glu Gln Ala Asp Ser Leu Glu Arg Ser Leu Glu Ala
1140 1145 1150

Glu Arg Ala Ser Arg Ala Glu Arg Asp Ser Ala Leu Glu Thr Leu Gln
1155 1160 1165

Gly Gln Leu Glu Glu Lys Ala Gln Glu Leu Gly His Ser Gln Ser Ala
1170 1175 1180

Leu Ala Ser Ala Gln Arg Glu Leu Ala Ala Phe Arg Thr Lys Val Gln
1185 1190 1195 1200

Asp His Ser Lys Ala Glu Asp Glu Trp Lys Ala Gln Val Ala Arg Gly
1205 1210 1215

Arg Gln Glu Ala Glu Arg Lys Asn Ser Leu Ile Ser Ser Leu Glu Glu
1220 1225 1230

Glu Val Ser Ile Leu Asn Arg Gln Val Leu Glu Lys Glu Gly Glu Ser
1235 1240 1245

Lys Glu Leu Lys Arg Leu Val Met Ala Glu Ser Glu Lys Ser Gln Lys
1250 1255 1260

Leu Glu Glu Ser Cys Ala Cys Cys Arg Gln Arg Gln Pro Ala Thr Val
1265 1270 1275 1280

Pro Glu Leu Gln Asn Ala Ala Leu Leu Cys Gly Arg Arg Cys Arg Ala
1285 1290 1295

Ser Gly Arg Glu Ala Glu Lys Gln Arg Val Ala Ser Glu Asn Leu Arg
1300 1305 1310

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Gln Glu Leu Thr Ser Gln Ala Glu Arg Ala Glu Glu Leu Gly Gln Glu
1315 1320 1325

Leu Lys Ala Trp Gln Glu Lys Phe Phe Gln Lys Glu Gln Ala Leu Ser
1330 1335 1340

Thr Leu Gln Leu Glu His Thr Ser Thr Gln Ala Leu Val Ser Glu Leu
1345 1350 1355 1360

Leu Pro Ala Lys His Leu Cys Gln Gln Leu Gln Ala Glu Gln Ala Ala
1365 1370 1375

Ala Glu Lys Arg His Arg Glu Glu Leu Glu Gln Ser Lys Gln Ala Ala
1380 1385 1390

Gly Gly Leu Arg Ala Glu Leu Leu Arg Ala Gln Arg Glu Leu Gly Glu
1395 1400 1405

Leu Ile Pro Leu Arg Gln Lys Val Ala Glu Gln Glu Arg Thr Ala Gln
1410 1415 1420

Gln Leu Arg Ala Glu Lys Ala Ser Tyr Ala Glu Gln Leu Ser Met Leu
1425 1430 1435 1440

Lys Lys Ala His Gly Leu Leu Ala Glu Glu Asn Arg Gly Leu Gly Glu
1445 1450 1455

Arg Ala Asn Leu Gly Arg Gln Phe Leu Glu Val Glu Leu Asp Gln Ala
1460 1465 1470

Arg Glu Lys Tyr Val Gln Glu Leu Ala Ala Val Arg Ala Asp Ala Glu
1475 1480 1485

Thr Arg Leu Ala Glu Val Gln Arg Glu Ala Gln Ser Thr Ala Arg Glu
1490 1495 1500

Leu Glu Val Met Thr Ala Lys Tyr Glu Gly Ala Lys Val Lys Val Leu
1505 1510 1515 1520

Glu Glu Arg Gln Arg Phe Gln Glu Glu Arg Gln Lys Leu Thr Ala Gln
1525 1530 1535

Val Glu Glu Leu Ser Lys Lys Leu Ala Asp Ser Asp Gln Ala Ser Lys
1540 1545 1550

Val Gln Gln Gln Lys Leu Lys Ala Val Gln Ala Gln Gly Gly Glu Ser
1555 1560 1565

Gln Gln Glu Ala Gln Arg Phe Gln Ala Gln Leu Asn Glu Leu Gln Ala
1570 1575 1580

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Gln Leu Ser Gln Lys Glu Gln Ala Ala Glu His Tyr Lys Leu Gln Met
1585 1590 1595 1600

Glu Lys Ala Lys Thr His Tyr Asp Ala Lys Lys Gln Gln Asn Gln Glu
1605 1610 1615

Leu Gln Glu Gln Leu Arg Ser Leu Glu Gln Leu Gln Lys Glu Asn Lys
1620 1625 1630

Glu Leu Arg Ala Glu Ala Glu Arg Leu Gly His Glu Leu Gln Gln Ala
1635 1640 1645

Gly Leu Lys Thr Lys Glu Ala Glu Gln Thr Cys Arg His Leu Thr Ala
1650 1655 1660

Gln Val Arg Ser Leu Glu Ala Gln Val Ala His Ala Asp Gln Gln Leu
1665 1670 1675 1680

Arg Asp Leu Gly Lys Phe Gln Val Ala Thr Asp Ala Leu Lys Ser Arg
1685 1690 1695

Glu Pro Gln Ala Lys Pro Gln Leu Asp Leu Ser Ile Asp Ser Leu Asp
1700 1705 1710

Leu Ser Cys Glu Glu Gly Thr Pro Leu Ser Ile Thr Ser Lys Leu Pro
1715 1720 1725

Arg Thr Gln Pro Asp Gly Thr Ser Val Pro Gly Glu Pro Ala Ser Pro
1730 1735 1740

Ile Ser Gln Arg Leu Pro Pro Lys Val Glu Ser Leu Glu Ser Leu Tyr
1745 1750 1755 1760

Phe Thr Pro Ile Pro Ala Arg Ser Gln Ala Pro Leu Glu Ser Ser Leu
1765 1770 1775

Asp Ser Leu Gly Asp Val Phe Leu Asp Ser Gly Arg Lys Thr Arg Ser
1780 1785 1790

Ala Arg Arg Arg Thr Thr Gln Ile Ile Asn Ile Thr Met Thr Lys Lys
1795 1800 1805

Leu Asp Val Glu Glu Pro Asp Ser Ala Asn Ser Ser Phe Tyr Ser Thr
1810 1815 1820

Arg Ser Ala Pro Ala Ser Gln Ala Ser Leu Arg Ala Thr Ser Ser Thr
1825 1830 1835 1840

Gln Ser Leu Ala Arg Leu Gly Ser Pro Asp Tyr Gly Asn Ser Ala Leu
1845 1850 1855

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Leu Ser Leu Pro Gly Tyr Arg Pro Thr Thr Arg Ser Ser Ala Arg Arg
1860 1865 1870

Ser Gln Ala Gly Val Ser Ser Gly Ala Pro Pro Gly Arg Asn Ser Phe
1875 1880 1885

Tyr Met Gly Thr Cys Gln Asp Glu Pro Glu Gln Leu Asp Asp Trp Asn
1890 1895 1900

Arg Ile Ala Glu Leu Gln Gln Arg Asn Arg Val Cys Pro Pro His Leu
1905 1910 1915 1920

Lys Thr Cys Tyr Pro Leu Glu Ser Arg Pro Ser Leu Ser Leu Gly Thr
1925 1930 1935

Ile Thr Asp Glu Glu Met Lys Thr Gly Asp Pro Gln Glu Thr Leu Arg
1940 1945 1950

Arg Ala Ser Met Gln Pro Ile Gln Ile Ala Glu Gly Thr Gly Ile Thr
1955 1960 1965

Thr Arg Gln Gln Arg Lys Arg Val Ser Leu Glu Pro His Gln Gly Pro
1970 1975 1980

Gly Thr Pro Glu Ser Lys Lys Ala Thr Ser Cys Phe Pro Arg Pro Met
1985 1990 1995 2000

Thr Pro Arg Asp Arg His Glu Gly Arg Lys Gln Ser Thr Thr Glu Ala
2005 2010 2015

Gln Lys Lys Ala Ala Pro Ala Ser Thr Lys Gln Ala Asp Arg Arg Gln
2020 2025 2030

Ser Met Ala Phe Ser Ile Leu Asn Thr Pro Lys Lys Leu Gly Asn Ser
2035 2040 2045

Leu Leu Arg Arg Gly Ala Ser Lys Lys Ala Leu Ser Lys Ala Ser Pro
2050 2055 2060

Asn Thr Arg Ser Gly Thr Arg Arg Ser Pro Arg Ile Ala Thr Thr Thr
2065 2070 2075 2080

Ala Ser Ala Ala Thr Ala Ala Ala Ile Gly Ala Thr Pro Arg Ala Lys
2085 2090 2095

Gly Lys Ala Lys His
2100

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(2) INFORMATION FOR SEQ ID NO:5:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 353 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(11) MOLECULE TYPE: DNA

(ix) FEATURE:

- (A) NAME/KEY: mRNA
- (B) LOCATION: 1..353
- (D) OTHER INFORMATION: /note= "ANTI-SENSE SEQUENCE TO PART OF THE MT1 MRNA TRANSCRIPT: N TERMINUS OF PROTEIN CODING SEQUENCE AND UPSTREAM 53 NUCLEOTIDES."

(ix) FEATURE:

- (A) NAME/KEY: misc feature
- (B) LOCATION: complement (298..300)
- (D) OTHER INFORMATION: /note= "MT1 INITIATION CODON SEQUENCE ON COMPLEMENTARY STRAND."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CTCAATTTTA ACTTGTTCTT GTTTTTCTCG TTGTGCAAGG CGAGCTGCAA CTTCTTCAGG	60
TGGTCGCTCC CTTATAGAAG ATGAGGATGC TTCTGAAAGT GCAGGTGTGG GTTTTCCTTC	120
ACCAATTTCA GGGTGATCAG TTTTTAAAGA TTCCTCAGGC TGAAGTCAG GGGCTGGGAC	180
CGACAGGGTA TCACCTGCTG CAGAAATAAT TTGAGCCGCT TCTGTAGGTG CTGTTGCTGA	240
AGCTGGAGTA TCTCCCTTTT GTTGTGGGAG TTGTGAGGCA GGCTGTTTAG ATTCTTTCAT	300
TACTTCTGAT AACTAGAGA TTTTGTAGTG ACCCGACTGA ATCGATTCT TTTG	353

(2) INFORMATION FOR SEQ ID NO:6:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 348 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(11) MOLECULE TYPE: DNA

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(ix)FEATURE:

- (A) NAME/KEY: mRNA
- (B) LOCATION: 1..348
- (D) OTHER INFORMATION: /note= "ANTISENSE SEQUENCE TO PART OF MT2
TRANSCRIPT: N TERMINUS OF PROTEIN CODING
REGION AND UPSTREAM 48 NUCLEOTIDES."

(ix)FEATURE:

- (A) NAME/KEY: misc feature
- (B) LOCATION: complement (298..300)
- (D) OTHER INFORMATION: /note= "MT2 INITIATION CODON SEQUENCE ON
COMPLEMENTARY STRAND."

(xi)SEQUENCE DESCRIPTION: SEQ ID NO:6:

CATGGTCATC	TTCGCCAGTT	CCAGCTCTGA	TCCCTCTAGC	ACCTTCTGTG	CAGATACCAG	60
GCGTTCG	GGG	GAAGAGGGAT	GTTTTCGATT	TTTCTGCAGA	AAACTGCACA	120
TCTCTCTGAC	ACCGGCTGCT	TCTTGATTG	CTGTCCCTCT	TCAGTGCCAT	GGATTCTGTC	180
AATGATCTTG	ATGAAGATGC	TGCAGTCCTG	GAGCTGCAGC	ACAGCCTCCA	CAGGGTCAGC	240
CACGTGTAGA	CTGTTACCCC	AAGAGAGGAG	TGCAGCCCCC	CGGGTGGCGT	GGAGTGTCAT	300
CTTGGTGATG	CCAGACAGTC	ACTCCAATGC	GCCTGTAATC	CCAGCTAC		348

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What is claimed is:

- 1 1. An isolated nucleic acid comprising the DNA
2 sequence of Seq. ID No. 1, including variants
3 thereof.
- 1 2. An isolated nucleic acid that hybridizes with the
2 DNA sequence of Seq. ID No. 1 under stringent
3 hybridization conditions.
- 1 3. A host cell transfected with the nucleic acid of
2 claim 1 or 2.
- 1 4. A vector comprising the nucleic acid of claim 1
2 or 2.
- 1 5. A protein or protein fragment encoded by the DNA
2 sequence of Seq. ID No. 1, including variants
3 thereof, in combination with an adjuvant.
- 1 6. A protein, produced by recombinant DNA in a host
2 cell and isolated from said host cell, said
3 recombinant DNA having the sequence of Seq. ID No.
4 1, including variants thereof.
- 1 7. A binding protein that binds to an epitope on the
2 protein of claim 6.
- 1 8. The binding protein of claim 7 wherein said
2 binding protein is an antibody or an antibody
3 fragment.
1

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- 1 9. A method of manufacturing an antibody for use in
2 the detection of abnormal cell types, the method
3 comprising the steps of:
4
- 5 a) combining a recombinantly-produced protein or
6 protein fragment encoded by the DNA of Seq. ID
7 No. 1 (or 3), including a variant thereof, with
8 an adjuvant to form a composition suitable for
9 injection into a mammal;
10
- 11 b) injecting the composition into a mammal
12 to induce antibody production in said
13 mammal against said recombinantly-
14 produced protein or protein fragment; and
15
- 16 c) isolating said antibody from said mammal.
- 1 10. The method of claim 9 wherein said step of
2 isolating said antibody from said mammal is
3 performed by isolating from said mammal a cell
4 producing said antibody.
- 1 11. A method of detecting an abnormal cell type in a
2 sample containing cells or cell nucleus debris,
3 the method comprising the steps of:
4
- 5 (a) contacting the sample with a binding
6 protein that recognizes an epitope on a marker
7 protein comprising an amino acid sequence
8 encoded by the DNA of Seq. ID No. 1 or 3 or a
9 variant thereof; and
10

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11 (b) detecting the presence in the sample of
12 said marker protein or a fragment thereof.

1 12. The method of claim 9 or 11 wherein said abnormal
2 cell type is a malignant cell type.

1 13. The method of claim 12 wherein said malignant cell
2 type is characteristic of a malignant bladder,
3 breast, prostate, lung, colon, ovary or cervix
4 cell type.

1
2 14. The method of claim 11 wherein said binding
3 protein is an antibody that binds specifically to
4 an epitope on said marker protein or protein
5 fragment.

1 15. The method of claim 14 wherein said antibody has a
2 binding affinity for said epitope greater than
3 10^5M^{-1} .

1 16. The method of claim 15 wherein said antibody has a
2 binding affinity greater than 10^7M^{-1} .

1 17. The method of claim 11 comprising the additional
2 step of quantitating the abundance of said marker
3 protein in said sample.

1 18. The method of claim 11 wherein said sample
2 comprises a body fluid.

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1 19. The method of claim 18 wherein said body fluid is
2 selected from the group consisting of serum,
3 plasma, blood, urine, semen, vaginal secretions,
4 spinal fluid, ascitic fluid, peritoneal fluid,
5 sputum, and breast exudate.

1 20. A method for determining the degree of cell death
2 in a tissue, the method comprising the steps of:
3
4 (a) contacting a sample with a binding protein
5 that recognizes an epitope on a marker protein for
6 cell death, said marker protein comprising an
7 amino acid sequence encoded by the DNA of Seq. ID
8 No. 1 or 3 or a variant thereof; and

9
10 (b) detecting the concentration of said marker
11 protein or protein fragment released from the
12 cells of said tissue, said marker protein or
13 protein fragment comprising an amino acid sequence
14 encoded by the DNA sequence of Seq. ID No. 1 or 3
15 or a variant thereof,

16
17 the concentration of said marker protein or
18 protein fragment detected being indicative of the
19 degree of cell death in said tissue.

1 21. The method of claim 20 comprising the additional
2 steps of:

3
4 c) repeating, at intervals, the steps of
5 detecting the concentration of said marker
6 protein or protein fragments thereof; and
7

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8 d) comparing said detected concentrations,
9 wherein changes in said detected
10 concentrations are indicative of the status of
11 said tissue.

1 22. The method of claim 20 for use in monitoring
2 change in the status of a disease or the efficacy
3 of a therapy, wherein a decrease in said detected
4 concentrations is indicative of a decrease in cell
5 death, and an increase in said detected
6 concentrations is indicative of an increase in
7 cell death.

1 23. the method of claim 20 wherein said tissue is
2 characteristic of breast, prostate, lung, colon,
3 ovary, bladder or cervical tissue.

1 24. A method of detecting an abnormal cell type in a
2 sample containing cells or cell nucleus debris,
3 the method comprising the steps of:
4
5 a) contacting the sample with a nucleic acid
6 that hybridizes specifically to an mRNA
7 transcript encoded by the DNA sequence of Seq.
8 ID No. 1 or Seq. 3, said transcript, when
9 translated, encoding the amino acid sequence
10 of Seq. ID No 1 or Seq. ID No. 3 or a variant
11 thereof; and
12
13 b) detecting the presence in the sample of
14 said mRNA transcript or a fragment or variant
15 thereof.

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- 1 25. The method of claim 24 wherein said abnormal cell
2 type is a malignant cell type.
- 1 26. The method of claim 25 wherein said malignant cell
2 type is characteristic of a malignant breast,
3 prostate, lung, colon, cervix or bladder cell
4 type.
- 1 27. The method of claim 24 wherein said nucleic acid
2 hybridizes with said mRNA transcript under
3 stringent hybridization conditions.
- 1 28. The method of claim 24 comprising the additional
2 step of quantitating the abundance of said
3 transcript in said sample.
- 1 29. Use of a molecule capable of binding to the mRNA
2 transcript or protein product of MT1 or MT2,
3 including variants thereof, for the manufacture of
4 a cancer therapeutic agent.
- 1 30. Use according to claim 29 wherein said cancer
2 therapeutic agent is for the treatment of breast,
3 prostate, cervix, ovarian, bladder, colon,
4 prostate or lung cancer.
- 1 31. Use according to claim 30 wherein said molecule is
2 an oligonucleotide complementary to at least a
3 portion of the DNA sequence of Seq. ID No. 1 or 3.

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1 32. Use according to claim 31 wherein said
2 oligonucleotide is a synthetic oligonucleotide and
3 comprises at least a portion of the sequence of
4 Seq. ID No. 5 or 6.

1 33. Use according to claim 29 wherein said molecule is
2 a member of a binding pair capable of binding MT1
3 or MT2 or a variant thereof substantially
4 irreversibly.

1 34. Use according to claim 33 wherein said member of
2 said binding pair binds MT1 or MT2 or a variant
3 thereof with an affinity greater than about 10^9
4 M^{-1} .

5
1 35. A synthetic oligonucleotide in admixture with a
2 pharmaceutical carrier for use in the manufacture
3 of a therapeutic agent, said synthetic
4 oligonucleotide comprising a sequence
5 complementary to at least a portion of the mRNA
6 transcript of MT1 or MT2 or a variant thereof.
7

1 36. The synthetic oligonucleotide of claim 35
2 comprising a sequence complementary to at least a
3 portion of the DNA sequence of Seq. ID No. 1 or 3
4 or a variant thereof.

1 37. The synthetic oligonucleotide of claim 35
2 comprising at least a portion of the sequence of
3 Seq. ID No. 5 or 6 or a variant thereof.

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1 38. The synthetic oligonucleotide of claim 35 being at
2 least 15 nucleotides in length.

1 39. A binding protein for use in the manufacture of a
2 medicament, said binding protein having a binding
3 affinity of greater than about 10^9 M^{-1} for the
4 protein encoded by the DNA of Seq. ID No. 1 or 3,
5 or a variant thereof.

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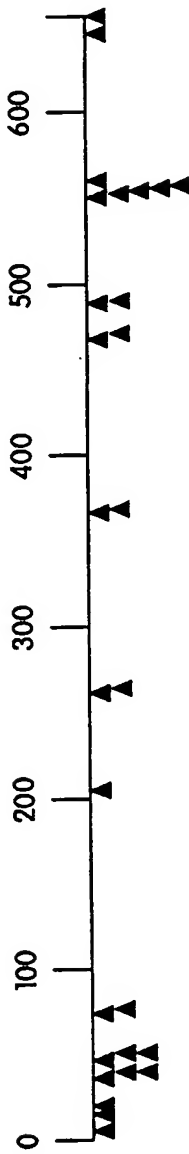


Fig. 1A

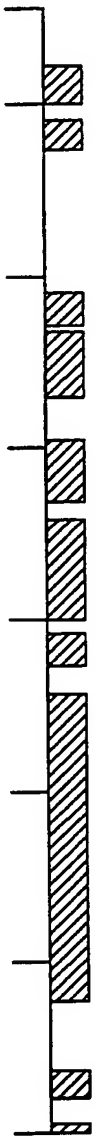


Fig. 1B

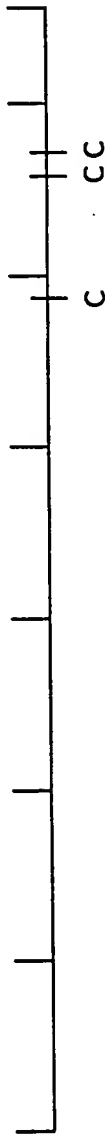


Fig. 1C

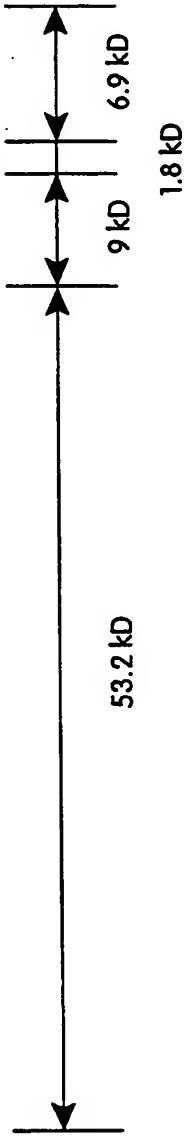


Fig. 1D

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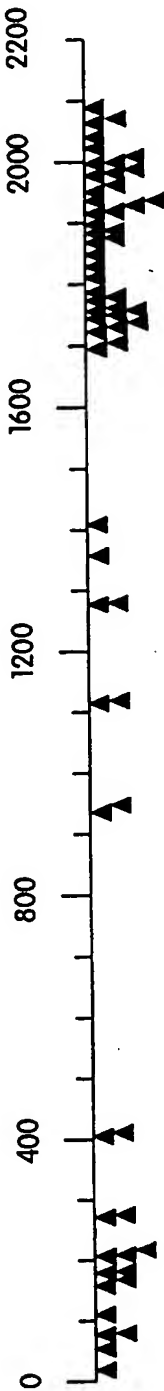


Fig. 2A



Fig. 2B

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FIG. 3.1

<u>SAMPLE</u>	<u>SAMPLE #</u>	<u>ANTIBODY COMBINATIONS</u>			
		<u>302-22</u> <u>302-18</u>	<u>302-33</u> <u>107-7</u>	<u>302-29</u> <u>302-18</u>	<u>302-29</u> <u>107-7</u>
NORMAL	1	0.0	0.0	0.0	0.0
NORMAL	2	0.0	0.0	0.0	0.0
NORMAL	3	0.0	0.0	0.0	0.0
NORMAL	4	0.0	0.0	0.0	0.0
NORMAL	5	0.0	0.0	0.0	0.0
NORMAL	6	0.0	0.0	0.0	0.0
NORMAL	7	0.0	0.0	0.0	0.0
NORMAL	8	0.0	0.0	0.0	0.0
NORMAL	9	0.0	0.0	0.5	0.0
NORMAL	10	0.0	0.7	1.2	0.0
NORMAL	11	0.0	0.0	0.0	0.0
NORMAL	12	0.0	0.0	0.0	0.2
NORMAL	13	0.0	0.7	0.0	0.3
NORMAL	14	0.0	1.3	0.0	0.6
NORMAL	15	0.0	5.3	0.0	1.7
NORMAL	16	0.0	1.4	0.0	0.4
NORMAL	17	0.0	2.2	0.0	1.0
NORMAL	18	0.0	2.0	0.0	0.0
NORMAL	19	0.0	3.0	0.0	0.4
NORMAL	20	0.0	2.3	0.0	1.3
NORMAL	21	0.0	3.9	0.0	0.6
NORMAL	22	0.0	8.2	0.0	1.3
NORMAL	23	0.0	4.0	0.0	0.8
NORMAL	24	0.0	4.3	0.0	0.7
NORMAL	25	0.0	9.1	0.0	0.6
NORMAL	26	0.0	5.9	0.0	0.2
NORMAL	27	0.0	20.6	0.0	6.0
NORMAL	28	0.9	2.2	0.9	0.7
NORMAL	29	1.4	5.0	0.0	1.0
NORMAL	30	1.4	3.5	1.4	1.2
NORMAL	31	1.9	10.1	3.9	1.0
NORMAL	32	2.1	3.3	0.0	6.3
NORMAL	33	2.8	1.5	0.0	0.0
NORMAL	34	4.1	6.9	6.6	0.8
NORMAL	35	4.2	0.0	5.4	0.0
NORMAL	36	11.0	1.2	5.6	0.0
BLADDER CA	37	0.0	0.0	0.0	0.0
BLADDER CA	38	36.7	1.6	0.0	0.0
BLADDER C	39	0.0	0.0	0.0	0.0
COLON CA	40	8.8	8.9	8.6	7.0
COLON CA	41	18.2	28.4	20.8	24.3
COLON CA	42	18.1	28.6	19.5	17.9
COLON CA	43	14.2	11.6	15.5	8.1
COLON CA	44	9.5	12.8	13.3	6.8
COLON CA	45	5.1	6.4	4.1	0.9
COLON CA	46	4.9	3.7	5.1	2.4

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FIG. 3.2

<u>SAMPLE</u>	<u>SAMPLE #</u>	<u>ANTIBODY COMBINATIONS</u>			
		<u>302-22</u> <u>302-18</u>	<u>302-33</u> <u>107-7</u>	<u>302-29</u> <u>302-18</u>	<u>302-29</u> <u>107-7</u>
COLON CA	47	30.8	28.3	65.3	27.3
COLON CA	48	96.2	17.5	82.4	20.2
COLON CA	49	3.3	4.7	0.0	0.0
COLON CA	50	10.1	11.7	8.3	10.3
COLON CA	52	2.4	5.7	64.7	0.0
COLON CA	53	6.7	5.1	5.5	0.5
COLON CA	54	5.1	6.0	1.3	1.8
COLON CA	55	3.9	13.1	7.1	2.3
COLON CA	56	62.4	9.6	52.4	5.8
COLOREC CA	57	14.0	58.2	15.2	41.3
ENDOMETRIUM C	58	7.6	10.3	106.0	6.8
ENDOMETRIUM C	59	2.7	4.7	1.8	1.9
ENDOMETRIUM C	60	7.9	9.4	8.2	7.1
LUNG CA	61	10.0	13.4	10.7	9.3
LUNG CA	62	9.5	11.9	11.0	7.9
LUNG CA	63	11.3	19.0	13.5	16.2
LUNG CA	64	6.5	16.7	8.5	7.8
LUNG CA	65	12.6	20.8	14.9	11.0
OVARY CA	66	14.3	21.1	17.4	16.9
OVARY CA	67	7.0	16.4	9.9	8.9
OVARY CA	68	8.9	11.6	11.5	8.3
PROSTATE CA	69	11.4	12.7	13.8	10.8
PROSTATE CA	70	2.0	4.9	2.5	2.8
PROSTATE CA	71	6.4	0.0	9.3	3.4
PROSTATE CA	72	5.4	15.4	6.3	7.0
PROSTATE CA	73	2.2	0.0	1.6	0.0

SUBSTITUTE SHEET

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FIG. 4

<u>TISSUE TYPE</u>	<u>ASSAY 1*</u>	<u>ASSAY 2**</u>	<u>ASSAY 3***</u>
Breast normal 90-247	NT#	500	1250
Breast normal 90-252	7574	2705	5024
Breast normal 90-254	NT	1513	2789
Breast normal 90-264	NT	0	1685
Breast normal 90-268	139	NT	432
Breast cancer 90-256	438	NT	2750
Breast cancer 90-275	2000	NT	9429
Breast cancer 90-287	20222	7333	8600
Cervix normal 90-279	2500	NT	12571
Cervical cancer 90-8083	12666	NT	70680
Colon normal 90-253	1009	NT	1689
Colon cancer 90-250	1450	NT	4275
Kidney normal 90-259	4250	NT	4275
Kidney cancer 90-289	2407	NT	5796
Liver normal	2154	614	202
Liver normal 90-451	NT	131	420
Liver cancer	2227	0	932
Met liver 90-403	NT	300	1133
Lung normal 90-248	4391	NT	6636
Lung normal 90-246	4200	NT	10000
Lung normal 90-107	NT	4166	388
Lung normal 90-118	NT	650	1200
Lung cancer 90-095	NT	5357	16077
Lung cancer 90-121	NT	>12000	40771
Ovarian cancer	8621	6517	2760
Ovarian cancer 90-260	6900	NT	20680
Ovarian cancer 90-291	2768	NT	5750
Ovarian cancer 90-291	NT	10909	14454
Uterine cancer 90-277	6574	NT	70684
Uterus normal 90-295	6574	NT	41444
average normal	3447	1284	5759
average cancer	9442	7069	26321

-
- * Assay 1 is 107.7 solid phase and 307.33 soluble phase.
 ** Assay 2 is 107.7 solid phase and 302.29 soluble phase.
 *** Assay 3 is 302.18 solid phase and 302.22 soluble phase.
 # NT means not tested.

INTERNATIONAL SEARCH REPORT

PCT/US 93/06160

International Application No

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) ⁶			
According to International Patent Classification (IPC) or to both National Classification and IPC			
Int.Cl. 5	C12N15/12; A61K31/70;	C12N15/11; A61K37/02;	C12Q1/68; A61K39/395;
			C07K13/00 G01N33/577
II. FIELDS SEARCHED			
Minimum Documentation Searched ⁷			
Classification System	Classification Symbols		
Int.Cl. 5	C12N ; G01N	C12Q ;	C07K ; A61K
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸			
III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹			
Category ¹⁰	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²		Relevant to Claim No. ¹³
X	J. CELL BIOL. vol. 116, no. 6, March 1992, ROCKEFELLER UNIVERSITY PRESS, NY, US; pages 1303 - 1317 C.H. YANG ET AL. 'NuMA: An unusually long coiled-coil related protein in the mammalian nucleus' cited in the application		9, 10
Y	see page 1304, right column, line 55 - page 1305, left column, line 27; figure 7 --- -/--		11-34, 36-38
<p>¹⁰ Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&" document member of the same patent family</p>			
IV. CERTIFICATION			
Date of the Actual Completion of the International Search		Date of Mailing of this International Search Report	
06 OCTOBER 1993		15. 10. 93	
International Searching Authority		Signature of Authorized Officer	
EUROPEAN PATENT OFFICE		HORNIG H.	

International Application No

PCT/US 93/06160

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	J. CELL BIOL. vol. 116, no. 6, March 1992, ROCKEFELLER UNIVERSITY PRESS, NY,US; D.A. COMPTON ET AL. 'Primary structure of NuMA, an intranuclear protein that defines a novel pathway for segregation of proteins at mitosis' cited in the application	9,10
Y	see page 1396, left column, paragraph 3 - right column, paragraph 1; figure 8 ---	11-34, 36-38
Y	J. CELL BIOLOGY vol. 115, 1991, ROCKEFELLER UNIVERSITY PRESS, NY,US; page 314A T.E. MILLER ET AL. 'Release of nuclear matrix proteins during apoptotic cell death' abstracts of papers presented at the thirty-first annual meeting of the american society for cell biology, Boston, Massachusetts, US; december 8-12,1991; Abstract no.1822; abstract ---	11-34, 36-38
Y	CANCER RESEARCH vol. 52, no. 2, 15 January 1992, WARVERLY PRESS, INC., BALTIMORE, MD, US; pages 422 - 427 T.E. MILLER ET AL. 'Detection of nuclear matrix proteins in serum from cancer patients' see page 425, right column, paragraph 4 - page 426, right column, paragraph 2 ---	11-34, 36-38
A	WO,A,8 703 910 (MASSACHUSETTS INSTITUT OF TECHNOLOGY) 2 July 1987 cited in the application the whole document ---	-
P,X	WO,A,9 309 437 (MATRITECH, INC.) 13 May 1993 cited in the application	7,8
Y	see page 21, line 24 - line 34 see page 29, line 13 - line 15 see page 34, line 1 - line 17; table I -----	11-23

ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO.

US 9306160
SA 76663

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 06/10/93

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		EP-A- 0250589	07-01-88
		JP-T- 63502484	22-09-88
		US-A- 4885236	05-12-89

WO-A-9309437	13-05-93	AU-A- 3123593	07-06-93

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